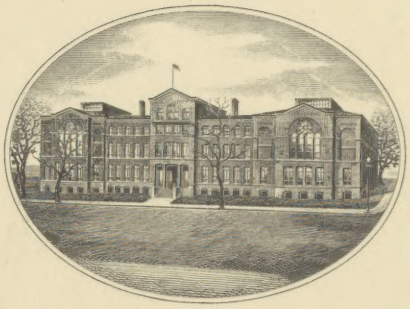

LABORATORY NOTES

IN

PHYSIOLOGICAL CHEMISTRY.

PART I.

BY

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P R E F A C E .

The following notes are temporarily placed in this form in order to save much time in dictation and writing. They cover the work given in the laboratory during the first four weeks of each course and are to be used in connection with the printed "DIRECTIONS".

F. G. NOVY.

March 29, 1897.

C O N T E N T S.

CHAPTER I.

F A T S.

PREPARATION OF PURE FAT.

1). Cut up 10g of subcutaneous pork fat, or of suet, into as small pieces as possible. Place in a small evaporating dish, 2 1/2-3 inches in diameter, and cautiously heat over a small flame stirring continually with a thermometer. Keep the temperature at 120°-130° * for about 10 minutes. Then strain through a small piece of muslin and squeeze thoroughly, receiving the clear fat in an evaporating dish(4 inch). Transfer the residue to a small mortor, add about 5cc of strong alcohol and rub up fine. Transfer the suspension now to a 75cc Erlenmeyer flask, rinsing out the mortor with several successive small portions of alcohol. Insert into the neck of the flask a stopper provided with a condensing tube about 24 inches long. Heat on the water-bath to boiling for 10 minutes. Then set aside and when the suspended particles have settled decant the clear alcohol into a small filter and receive the alcoholic filtrate in the evaporating dish containing the bulk of the fat. To the insolu ble residue remaining in the flask, add 20cc of ether, insert condensing tube, and cautiously boil on the water-bath for about 5 minutes. Then transfer the entire contents of the flask to the filter previously used and receive the ethereal filtrate in the evaporating dish. Finally wash the residue with a little ether, then squeeze out most of the ether. Open the filter and allow the remaining ether to spontaneously evaporate. Save this yellowish residue of connective tissue for a subsequent experiment.

The evaporating dish now contains the strained fat, also the alcoholic and ethereal filtrates. Place it on an evaporating dish and heat till all the alcohol, ether and water have been driven off and only the pure fat remains.

The student will use for the above experiment, alternately, pork fat and suet.

2). Place a little of the pure fat obtained above on a slide, cover and examine under the microscope. Observe that the little round bodies are composed of crystals. These are more distinct in the beef fat.

3). Transfer a piece of fat, size of a pea, by means of a glass rod to a test-tube. Add 5cc of a mixture of equal parts of alcohol and ether and warm gently till dissolved. Then set aside for an

* The temperature given in the work are Centigrade.

hour or more and when a deposit forms transfer some of it by means of a pipette to a glass slide, cover and examine under the microscope. Sketol, the crystals obtained thus from tallow and from lard. Which of these two fats crystallizes most rapidly?

4). Transfer a piece of fat to a test-tube, add 5cc of alcohol and heat till dissolved. Then introduce a strip of blue litmus paper, or add a drop of an aqueous solution of litmus. Better still to some of the alcoholic solution add a drop of alcholic rosolic acid, a yellowish color indicates an acid reaction. What is the reaction of normal fat? Why do fats become rancid on standing for some time?

5). Place a small piece of fat on a filter paper and warm gently over a flame, or on a heated plate till the fat melts and is absorbed. Note the transparent condition of the paper.

6). Rub up thoroughly in a mortor a piece of fat with some $KHSO_4$. Transfer the mixture to a dry test-tube and heat cautiously. The peculiar irritating odor or sensation is due to acrolein or acrylic aldehyde, which is formed by dehydration from the glycerin of the fat.

Glycerin, $CH_2OH.CHOH.CH_2OH$.
Acrolein, $CH_2:CH.COH$.

7). To a small piece of fat in the test tube add about 10cc of a semi-saturated solution of sodium carbonate, warm and shake thoroughly. The liquid becomes milky but on standing most of the fat collects on the surface. The liquid below shows but slight cloudiness. Neither emulsion, solution or saponification has taken place.

8). Saponification.--Melt the fat that is left from the preceeding experiments and transfer it to a 150cc Erlemmeyer flask. Then add 20-30cc of alcohol and 3g of KOH. Insert a condensing tube and heat on the water-bath for about a half an hour. Saponification takes place rapidly. To ascertain if the change is complete pour a little of the alcoholic fluid into a few cc of water*. The liquid must remain clear. If it becomes cloudy it is due to oil drops and shows that the saponification is incomplete. The solution eventually contains soap, glycerine, and excess of alkali and alcohol.

9). Separation of the Fatty Acids.--To about 100cc of water in a small beaker add 3cc of H_2SO_4. Then warm to about 50°. Pour the soap solution, gradually, and with constant stirring, into the warm acid liquid. The fatty acids are set free and rise to the surface forming a clear, oily liquid. Place the beaker on a water-bath and heat till the aqueous liquid below the fatty acid

* Distilled water is meant in all cases unless otherwise stated.

layer becomes almost clear, and all the fatty acid has risen to the surface. At the same time prepare some boiling water.

Then transfer the contents of the beaker to a small filter, previously moistened with hot water. The fatty acids, while still liquid, are washed on the filter with hot water (10-12 times) till the wash water ceases to give with Ball 2 a test for H_2SO_4. Collect the aqueous filtrate and wash-water and set it aside to be examined later for glycerine.

The funnel containing the washed fatty acids is now placed upright in a small beaker containing cold water, the level of which should correspond to that of the fatty acid on the filter. The fatty acids solidify. The product thus obtained is a mixture of oleic acid, $C_{18}H_{34}O_2$, palmitic acid $C_{16}H_{32}O_2$, and stearic acid $C_{18}H_{36}O_2$. Commercial stearin which is used in the manufacture of candles is a mixture of palmitic and stearic acids.

10). Reactions with Fatty Acids.--To some of the sokid fatty acid in a test-tube add about 10cc of strong alcohol and warm till the acid sissolves. Divide into three portions. To one portion add a drop of rosolic acid; to another portion 1-2 drops of aqueous litmus solution; to a third portion a strip of blue litmus paper. What is the reaction and which reagent is more delicate?

11). To a portion of the fatty acid apply the test given under Exp. 5. Why do fats, fatty acids, glycerin water, etc. render paper more transparent?

12). To a portion of the fatty acid apply the test given under Exp.6. What is the result?

13). To about 10cc of semi-saturated Na_2CO_3 solution add a small portion of the fatty acids and heat. An effervescence results, carbonic acid is given aff. The fatty acids dissolve and a sodium soap is formed. Place the tube in a beaker of cold water ,a soap jelly results.

Warm the tube again till the contents are liquid, then add a few drops of cottonseed or olive oil and shake. An opalescent liquid, or emulsion forms. Transfer a drop of this to a slide, cover and examine under the microscope. Note the highly refracting fat globules.

14). Place some of the fatty acid in a small beaker, add about 50cc of water and warm gently till the fatty acids mel . Then add dilute NaOH, drop by drop, and stir thoroughly after each addition. Continue addition of alkali till the fatty acid just dissolves. With this sodium soap solution make the following tests:

a). To some of the solution add a few drops of $CaCl_2$ solution.

An insoluble Calcium stearate etc. forms. This calcium soap is formed when hard water is used with soap.

b). To another portion add some lead acetate and warm gently. The white sticky precipitate which forms is lead soap. It is known and used medicinally as lead plaster.

15). Separation of Glycerine.--The combined aqueous filtrate and washings from the fatty acids, should, if oily globules are present, be filtered through a wet filter. The filtrate is then carefully neutralized with NaOH and concentrated in an evaporating dish, first over a flame, and finally on a water-bath almost to dryness. To the residue add about 25cc of alcohol, stir thoroughly and allow the mixture to stand for 1/4-1/2 hour, then filter. To the residue add another portion of about 15cc of alcohol, stir well and transfer this washing to the filter. Evaporate the alcoholic filtrate and washings on the water-bath to dryness. Take up the residue with about 15cc of absolute alcohol and transfer this entire mixture to a large test-tube; then add an equal volume of ether cork and shake and set aside in a beaker of cold water for about a half an hour. Filter off the salts which are thus thrown out of solution and cautiously evaporate the alcoholic-ethereal liquid on a slightly warmed water-bath. A syrupy residue, glycerine remains.

16). Taste the yellowish syrup that is left.

17). Place a drop of the residue on a slide and add a little powdered borax. Then touch the mixture with a platinum wire and place this in a Bunsen flame. Note the green color.

18). Mix a drop or two of the syrup with some powdered $KHSO_4$ and heat in a dry test-tube. Compare the result with that obtained in experiment 6 and 12.

CHAPTER II.

CARBOHYDRATES.

In this group are usually placed those substances which contain H and O in the same proportion as in water (2:1) and 6 carbon atoms or a multiple of 6. Recent investigations have shown that we may have carbohydrates containing from 4 to 9 or more carbon atoms. There are furthermore unquestionable sugars . . as rhamnose, which do not have H and O in the proportion of 2 to 1.

Carbohydrates are present in comparatively small amounts, either free or as constituentsof certain complex proteids, in the animal body. They constitute, however, the greater part of the solids of plants, just as proteids make up the greater part of the animal body. They are aldehyde or keton derivatives of certain alcohols.

The following condensed classification is adapted from Tollens.

I. MONOSACCHARIDES OR GLYCOSES.
This includes besides others, pentoses, $C_5H_{10}O_5$, and hexoses, $C_6H_{12}O_6$, such as dextrose, laevulose, and rhamnose, $C_6H_{12}O_5$.

II. DI-SACCHARIDES, OR SACCHAROSES, $C_{12}H_{22}O_{11}$.
Cane-sugar, milk sugar, maltose, iso-maltose.

III. POLYSACCHARIDES.
A few of these compounds are crystallizable, but most of them are amorphous. The latter group includes pentosanes, which have the same relation in pentoses as starch bears to glucose. Also, starch, and its derivitives amylodextrin, erythrodextrin, and achrodextrin. Also glycogen, dextran and others, cellulose.

IV. MANNITE, $C_6H_{14}O_6$. The compounds of this group are related to the true carbohydrates.

V. INOSITE, $C_6H_{12}O_6$. This is a derivative of hexamethylene C_6H_{12}.—

As shown from the following formulae dextrose or glucose contains an aldehyde group whereas laevulose or fructose contains a ketone group.

Dextrose= $CH_2OH.CHOH.CHOH.CHOH.CHOH.CHO$.

Laevulose= $CH_2OH.CHOH.CHOH.CHOH.CO.CH_2OH$.

On treatment with nascent hydrogen the aldehyde or ketone group is readily reduced to the corresponding alcohol group CH_2OH, or $CHOH$. Mannite $C_6H_{14}O_6$. The pentose in a similar way yield correspondinf pentites.

The monosaccharides, like aldehydes, readily reduce salts of silver, copper, mercury, ect. It should be remembered that other substances, as lactose, maltose, glucuronic acid, alkapton, also reduce.

PENTOSES, $C_5H_{10}O_5$.

In plants these are substances pentosanes, which yield on hydration pentoses just as starch on similar treatment yields glucose. A pentose has been met with in the decomposition of glycoproteid obtained from the pancreas. It has been found recently in several urines; also in the urine of natural and artificial diabetes.

The pentoses are strong reducing agents, but are not fermentable by yeasts. With phenylhydrazin they yield osazons which melt at 157°-160°. On distillation with hydrochloric acid they yield furfurol which colors aniline acetate paper bright red.

HEXOSES, $C_6H_{12}O_6$.

Cane-sugar, $C_6H_{22}O_{11}$, which yields dextrose and laevulose, $C_6H_{12}O_6$, or hydration can therefore be considered as an anhydride of these hexoses. The hexoses, dextrose and laevulose, are widely distributed in plants, especially in acid fruits; and puthermore readily form on hydration of starch, cane-sugar, glucosides as phloridzin, etc. Another hexose, galactose, results on hydration of lactose, and other carbohydrates, and also of cerebrin.

The three hexoses mentioned are fermentable by yeast. On heating with dilute mineral acids they yield laevulinic acid, $C_5H_8O_3$, humus substances.

Dextrose or glucose, also known as grape-sugar or starch-sugar is formed during digestion. It is present in small amount, 0.1-0.2%, in the blood; in still less amount in normal urine. In diabetes it is present sometimes in considerable quantities as the characteristic constituent of urine. After the digestion of large quantities of cane-sugar, lactose or glucose a reducing substance appears in the urine (alimentary glycosuria). A part of the cane-sugar may appear as such in the urine. Glucose appears in the urine after administration of phloridzin, uranium salts, hydrocyanic acid; also when the oxygen supply is diminished and in CO poisoning. Reducing subatances, presumably glucose, and formed on the decompostion of cartilage, nucleinic acid, paranuclein, nucleoproteid of the pancreas etc.

It can be obtained as minute crystals which are either anhydrous or contain one molecule of water. It is only about 3/5 as sweet as cane-sugar. It is soluble in about an equal part of water; insoluble in absolute alcohol. The solutions are dextro-rotatory. The melting-point is at 144-146°; above 200° caramel forms.

GLUCOSE.

In the following experiments, unless otherwise indicated, a 2% solution of glucose is employed.

1). Molish's reaction.--To about 1/2 cc of the dilute sugar solution add 1-2 drops of an alcoholic solution (15%) of α-napthol. Then add slowly about 1cc of concentrated H_2SO_4 so that it runs down the side of the inclined tube and forms a layer. A beautiful reddish violet ring forms at the zone of contact.

This is a general reaction due to the formation of furfurol and is given by all carbohydrates. Apply this test to some normal urine; and to urine diluted with 5 parts of water. If the test is given by the latter it indicates that the carbohydrates of the urine are increased.

2). Place some of the dry glucose in a tube and heat gently over a flame. It melts, then turns yellow and finally dark-brown. The peculiar odor is that of burnt-sugar. Allow the tube to cool, then add water and warm slightly. Note the dark yellow or brownish color of the solution. Caramel is a harmless coloring matter and is employed extensively for coloring liquors, vinegars, etc.

3). To some dry glucose add cold, concentrated H_2SO_4 and let stand. The liquid remains colorless or at most is light yellow. Distinction from cane-sugar. See experiment 3 under cane-sugar. After comparing this with the corresponding experiment with cane-sugar, gently heat the glucose tube. It promptly blackens due to humin substances. Laevulinic acid is formed at the same time .

4). To the sugar solution add some strong KOH solution and heat. The liquid becomes yellow, then dark-brown. The sugar undergoes oxidation in alkaline solutions. With solid KOH the reaction is sometimes violent because of the heat generated in the reaction.

This test when applied to urine is known as Moore-Heller's test. In that case the precipitate that forms is due to earthy phosphates. The test is not particularly delicate and is certainly not reliable since the substances may yield dark solutions under similar conditions, namely alkapton, lactose, maltose, etc.

Compare this reaction with experiment _____ under cane-sugar.

5). To the sugar solution add 1/2 volume of Na_2CO_3 solution, then 1-2 drops of a freshly prepared solution of potassium ferricyanide, and boil. The liquid becomes colorless--due to a reduction of the salt to a ferrocyanide.

6). To the sugar solution add a little ammoniacal silver nitrate

and a few drops of KOH and warm gently. A mirror of metallic silver forms, expecially if the solutions are dilute. The silver has been reduced.

The ammonical silver nitrate is prepared by adding ammonium hydrate to the silver nitrate till the precipitate just appears.

7). To the sugar solution add one drop of a freshly prepared solution of sodium indigo sulphate, also add a little Na_2CO_3 solution and heat. The blue color changes first to violet then to red, yellow and finally the liquor is colorless. The indigo has been reduced to indigo-white. Cool the tube under the hydrant and shake. The indigo-whits is oxidized to indigo blue. On heating again the blue is again reduced. Litmus and other coloring agents are reduced in a similar manner.

The following reactions should be applied side by side, to the aqueous solution of glucose and to diabetic urine.

8). Trommer's Test.--Render the solution or urine strongly alkaline with KOH and boil then add a few drops of copper sulphate solution and warm again a reddish yellow precipitate of cuprous oxide forms. If excess of copper be added, the copper hydrate precipitate will mask small amounts of the red precipitate. If too little copper has been added a white precipitate of uric acid and nuclein bases (alloxuric bodies) forms.

8a). Fehling's Test.--Boil some Fehling's solution in a test-tube and then add the sugar solution or the suspected urine and boil. Cuprous oxide is thrown down. The urine if strongly acid should be rendered alkaline. This is the test commonly employed when examining for sugar in the urine. It should be remembered that it is not an absolute test since the urine, in rare cases, may contain other reducing substances(alkapton). A small amount of sugar may, moreover, escape detection since the cuprous oxide may be held in solution by creatinin and other urine constituents.

It sholud furthermore be remembered that Fehling's solution deteriorates on keeping, so that on heating the solution itself a red precipitate of cuprous oxide may form. It is advisable therefore to keep the two constituents of Fehling's solution in separate bottles and to mix equal volumes just before use.

Pavy's solution, employed for the same purpose is a solution of copper hydrate in ammonium chloride.

9). To some Barfoed's solution add some glucose solution and boil. The cuprous oxide precipitate forms. Milk sugar, cane-sugar, maltose and dextrin do not reduce this solution.

Barfoed's reagent is an acid solution of copper. It is prepared by dissolving 1 part of copper acetate in 15 parts of water. To 200cc of this solution, ~~5cc of a 28% acetic acid solution is added.~~

10). Böttger's Test.--Render the specimen alkaline with sodium or potassium hydrate, then add a minute quantity of basic bismuth nitrate--a black color or precipitate due to rëduced bismuth, forms. Albumin if present must be removed.

10a). Nylander's Test.-- Dissolve 10.33g sodium hydrade in 100cc of water; add 2g of basic bismuth nitrate, and 4g Rochelle salts; warm and filter; This reagent keeps better that Fehling's solution.

To 10 volumes of the sugar solution or urine add one volume of the reagent and boil 2-3 minutes. Then let stand for 10-15 minutes.

Concentrated urines may become blackish with this reagent; if chrysophonic acid is present in the urine this may also occur. On the other hand the reaction is more delicate than Fehling's solution, whereas pointed out, small amounts of cuprous oxide may be held in solution.

Alkaline solutions of mercury salts are also employed in testing for glucose (Knapp, Sachsse).

11). Phenyl-hydrazin Test.--Phenyl-hydrazin on heating with sugar forms phenyl-glycosazon [illegible] $C_6H_{10}O_4(N_2H.C_6H_5)_2$. This forms bundles of yellow heedles which melt at 204-205°.

$$C_6H_{12}O_6+2C_6H_5.N_2H_3=C_{18}H_{22}N_4O_4+2H_2O+2H.$$

Application to the urine.--Place in a small beaker about 50cc of the clear urine add 1-2 g of phenylhydrazinc hydrochloride and about 2-4 g of sodium acetate, cover with a watch-glass and warm on the water-bath for 1/2--1 hour, then turn off the light and allow it to cool on the water-bath. Examine under the microscope the deposit which forms. If amorphous, or if it is desirable to purify the crystals, dissolve on the filter in hot alcohol. To the filtrate add water and boil till the alcohol is expelled--on cooling the characteristic yellow crystals appear. Filter, wash, dry and determine the melting point.

The phenyl-hydrazin reaction with sugars is of very great importance in their identification. It forms with sugars, when heated sufficiently long on the water-bath, osazones. The various sugars yield therefore corresponding osazones which are yellowish, and fdiffer in crystalline form, melting-point, solubility, and optical behavior. The determination of the melting-point is especially valueable.

12). Fermentation test.--Rub up some of the solution or of the suspected urine with a little yeast. Fill the mixture into a large, wide test-tube provided with a perforated stopper through which passes a tube bent into a U shape--the free arm being longer than the one that passes through the cork. Care should be taken to likewise fill the tube so that no air is present in the test-tube

when it is inverted. Set the tube aside in an inverted position in a warm place for 24 hours and observe the accumulation of gas.

When the fermentation is completed place the tube in an upright position in a dish of water, remove the stopper and by means of a bent pipette introduce a little potassium hydrate solution. What is the result?

$$C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2.$$

Under the influence of certain bacteria it readily undergoes lactic acid, lutyric acid or viscous fermentations.

Laevulose, also known as fruit sugar or frutose occurs, as before indicated widely distributed in the plant kingdom. It is also present with dextrose in honey. While starch on hydration yields dextrose, there are analogous substances, as inulin, $C_6H_{10}O_5$, which on similar decomposition yield laevulose. In exceptional cases it has been met with in the urine of diabetes. When administered in diabetes a part may be changed to glucose and to glycogen, and a part may be eliminated as such (Haycraft).

This sugar crystallizes with great difficulty and for that reason it is ordinarly met with as a thin syrup. It is readily soluble in water, insoluble in cold absolute alcohol. The solutions are laevorotatory.

The rotation is greater, and in opposite direction, than that of cane-sugar. Hence on hydration of cane-sugar the resulting mixture is laevo-rotatory, and is therefore called invert-sugar. Inversion, as applied to complex carbohydrates, is synonymous with hydration.

Like glucose it reduces readily metallic oxides; is fermented by yeast and forms the same osazon.

Galactose which forms with dextrose on the hydration of milk-sugar, and other carbohydrates, also of cerebris. It crystallizes in needles or plates which melt at 168°. It is dextro-rotatory. It reduces Fehling's solution and is said to ferment with yeast. It forms an osazone which melts at 193°, On oxidation it yields mucic acid--distinction from dextrose. The origin of galactose as a constituent of milk-sugar is not known. It may be derived from antecedents in the plant food, and on the other hand may be formed from glycogen or even glucose in the body.

CANE-SUGAR, $C_{12}H_{22}O_{11}$.

Saccharose, Sucrose.- This sugar is widely distributed in plants in the leaves of which, under the influence of light and possibly of chlorophyll, it is formed. It is then transported to different parts of the plant and may be stored up in the roots as in the case of beet root, or in the stalk, as in sugar cane. In acid liquid it very readily undergoes inversion and for that reason it is not present in strongly acid fruit juices but is represented

there by dextrose and laevulose. In moderately acid fruits as nuts, apples, melons, bananas, sweet oranges, it is present as such with more or less glucose. The cane-sugar which is removed from the flower by the bee becomes almost wholly inverted when made into honey.

It forms large nono-clinic crystals which dissolve in 1.5 parts of water at 20°. The solution is strongly dextro-rotatory. It melts at 160° and on further heating it yields caramel. It is decomposed by dilute acids, slowly in the cold, very rapidly on heating. The change is as follows:

$$C_{12}H_{22}O_{11} + H_2O = C_6H_{12}O_6 + C_6H_{12}O_6.$$
Dextrose Laevulose.

This hydration is also brought about by many ferments, such as the invertin of yeast.; by bacteria and moulds; also by the acid gastric juice but not by the pancreas. Once inverted the resultant invert-sugar is readily subject to various fermentations such as alcohol, viscous, lactic acid, etc.

Before inversion it is strongly dextro-rotatory and does not reduce Fehling solution, After inversion it is less dextro-rotatory, or even laevo-rotatory, and reduces Fehling solution. With phenyl hydrozin it does not form a corresponding osazon, but does form, owing to inversion, the phenylglycosazon. This behavior and the non-reduction of metallic oxides distinguishes cane-sugar from maltose and lactose. The latter therefore still allow the aldehyde character.

Apply the following reactions, with the exception of 2 and 3, to a 2% aqueous solution of cane-sugar, and compare these, side by side, with the corresponding reactions of glucose.

1). Molisch's Reaction.- Apply as given in Ex. 1, under glucose.

2). Caramel reaction.- Apply as given in Ex. 2, under glucose.

3). Sulphuric acid reaction.- Apply as given in Ex. 3 under glucose. The cold acid in a few minutes colors yellow, the n becoms black,--distinction from Dextrose. Mumin substances are formed.

4). Potassium hydrate reaction. Apply as given in corresponding test under glucose and carefully note the difference.

5). Apply Fehling's solution as in Ex. 8a under glucose.

6). Test with Barford's reagent, as in Ex. 9 for glucose.

7). Test with Nylander's reagent, as in Ex. 10a under glucose.

8). Apply the fermentation test as in Ex. 12 under glucose and compare the rapidity of fermentation with that of glucose.

9). Place 50 cc. of tje cane-sugar in a small beaker, add 6-8 drops of concentrated HCl and boil for 2-3 minutes. Then cool, tender alkaline with sodium or potassium hydrate. To this solution now apply tests 4, 5, 6, 7, as given above. Note the results.

LACTOSE, $C_{12}H_{22}O_{11} + H_2O$.

Lactose, or milk sugar, occurs probably in the milk of all animals. The amount present varies from 3-5-6%? It has been found in the urine during the later stages of pregnancy and immediately after birth. It is said to occur in one plant.

It forms large rhombic crystals which are soluble in 6 parts of cold water and in 2 1/2 parts of boiling water. The solution is

dextro-rotatory. When heated to 170-180° it forms lacto carmel, $C_6H_{10}O_5$; melts at 203.5°. On heating with acids hydration takes place according to the equation:

$$C_{12}H_{22}O_{11} + H_2O = C_6H_{12}O_6 + C_6H_{12}O_6.$$
Galactose. Dextrose.

On further heating with acids, humin and formic acid and laevulinic acids form. On oxidation with nitric acid inversion first takes place as above, and then the galactose is oxidized to mucic acid whereas the dextrose forms saccharic acid. It reduces Fehling's solution but is only 2/3 as strong as dextrose, Unlike the latter it is not fermented by yeast. Bacteria readily bring about lactic acid fermentation. In kephir and Kumyss the sugar is changed to alcohol and lactic acid.

With phenylhydrozin it combines to form a lactosazon which crystallizes on cooling as round aggregates of yellow needles which melt at 200°. Its behavior to cold concentrated sulphuric acid and to alkalies also serves to distinguish it from dextrose and cane-sugar respectively. Alkalies yield lactic acid and pyrocatechin.

To a 2% solution of lactose apply the tests 1-8 as given under cane-sugar. For the preparation of milk-sugar see MILK.

MALTOSE, $C_{12}H_{22}O_{11} + H_2O$.

This sugar is formed by the action of the germent diastase, contained in malt, on starch. It is also formed by the ferments of the saliva, pancrea and liver. The formation of dextrin precedes that of maltose. When starch is heated with H_2SO_4 maltose is temporarily produced. Consequently crude glucose and glucose syrup will contain maltose in small amounts.

It forms fine white needles, grouped in little masses. It is soluble in water and in dilute alcohol. On oxidation with nitric acid it yields saccharic acid. On heating with sulphuric acid it yields two molecules of dextrose. This change is also accomplished by ferments.

$$C_{12}H_{22}O_{11} + H_2O = C_6H_{12}O_6 + C_6H_{12}O_6.$$

Like dextrose it is easily fermented by yeast, and readily reacts with potassium hydrate, and with Fehling's solution. It reduces the latter more weakly than dextrose, 10 cc. of Fehling's solution represents 77.8 mg. maltose. The reaction with Barfoed's reagent serves to distinguish it from dextrose. With phenylhydrazin it forms an osazon—Maltosazon. This forms yellow separate needles which melt at 206°. It dissolves or can hold in solution ferric hydrate. It is dextro-rotatory.

Iso-Maltose, is an isomer of maltose. It is amorphous and is formed by the action of acids and ferments on starch. It has been prepared synthetically from glucose by the action of concentrated HCl. Unlike maltose it is more difficultly fermentable, and forms an osazon which melts at 153°. It readily reduces Fehling's solution. It is converted by diastase into maltose.

The relation of the three di-saccharides can be seen from the following:

Cane sugar + H_2O = glucose + laevulose.

Milk-sugar + H_2O = glucose + galactose.

1). To 100 cc. of boiling water add 10 g. of starch and still till an even starch paste forms. Then cool to 60° and add 1 g. of powdered malt. Immerse in a water-bath at 60° for one hour. At intervals of 10 minutes test 1-2 ccs of the liquid with iodine for dextrin (see page). Then boil and filter. Evaporate the filtrate to a thick syrup and set this aside for several days to crystallize. The addition of a thread, or of a crystal of maltose will favor crystallization. Note the taste of the syrup.

To the remaining 1/2 of the filtrate apply the tests 4-8 inclusive as given under cane-sugar.

STARCH $(C_6H_{10}O_5)_n$.

Starch, or amylum, is a highly complex carbohydrate and the value of n in the above formula is not determined. It is placed by some at as high as 200. Starches are also known as glucosins since on hydration they yield as a final product glucose or dextrose, whereas the inulins or laevulans, which correspond to starch, yield laevulose. The inulins are comparatively rare, whereas starch is a most widely distributed plant constituent. It is evidently formed from CO_2 by chlorophyll in the presence of water. In plants the excess of sugar is stored up as starch, while in animals it is stored up as glycogen. In the body of animals starch can unquestionably be converted into and deposited as fats. It is known that bacteria acting on starch can give rise to certain fatty acids.

Starch is containedin the so-called starch-granules which have a characteristic appearance and can be readily recognized under the microscope. The form of the granules as obtained from one plant differs from that obtained from other plants, The size of the granules varies greatly even in starch of the same variaty. The starch proper is deposited in these granules in layers around one or more nuclei. Some cellulose is present. Frequently, as a result, concentric rings will be observed in the starch granule.

On heating to 150-170° it becomes yellowish, and also soluble in water; that is, dextrin is formed. Commercial dextrin, which is used extensively as a mucilage, is prepared in this way.

Starch is insoluble in cold water. In the presence of chloride of zinc and other salts it swells up and dissolves. On heating with water to 60-70° it swells to a paste but does not form a true solution. At a higher temperature it does dissolve forming soluble starch and hydrolytic products described below. With glycerin, especially on heating, it forms soluble starch. On heating with dilute acids it dissolves readily, or is rather hydrated forming soluble products. The final product of the action of an acid is dextrose. HCl acts more rapidly than H_2SO_4. Diastatic enzymes, such as are contained in malt, saliva, pancreas, dissolve starch forming a number of intermediate products and finally maltose, not dextrose. Starch is not fermented by yeast but is affected by bacteria, such as lactic acid and butyric acid bacilli;also by moulds. Nitric acid first inverts starch, then oxidizes the products of saccharic, tartaric, and oxalic acids.

The products formed by the hydration of starch, brought about by water under pressure, by acids, or by ferments, are presented in the following table:

Starch	Iodine,	blue.	Fehling, -0	Tasteless
Soluble Starch	"	"	" -0	"
Erythrodextrin	"	red	" + very slight.	"
Achroodextrin	"	0	" + slight	"
Maltodextrin	"	0	"	Sweetish.
Isomaltose	"	0	" +	Sweet.
Maltose	"	0	" + Barfoed,0	"
Dextrose	"	0	" + " ,+	"

1). Examine microscopically and sketch the granules of the following starches: potato wheat, buck-wheat, corn, arrow-root and rice. Note the shape of granules, the number of rings if any, and the cleft or hilum.

2). Place a little starch in a test-tube add water and shake thoroughly, then filter, To the filtrate add a drop of iodine solution. No color is formed, since starch is insoluble. Add a drop or two of iodine to the residue on the filter--a blue color results.

3). Soluble starch.--Place 100cc of water in a beaker and boil then add 1g of powdered starch and continue boiling for 2-3 minutes stirring constantly. A starch paste forms.

4). Place some of the starch solution obtained in experiment 3 in a tube and add a drop of iodine so,ution. A deep blue color results. Now heat the ontents of the tube, the color disappears, to reappear on cooling. The blue dolor is due to the so called starch iodine, which possesses a variable composition.

5). To some of the starch solution add excess of tannic acid. A yellowish white precipitate forms or the liquid becomes highly opaque.

6). Boil some of the starch solution with Fehling's solution. No reaction takes place.

7). To about 50cc of the starch solution in a beaker add 1/2cc of H_2SO_4, cover with a watch-glass and boil for 15 minutes. Replace the water that may be lost by evaporation. Now place some of the liquid in a tube, render alkaline with sodium or potassium hydrate, add some Fehling's solution and boil. If no reduction takes place, continue heating the ontents of the beaker for another 15 minutes and test as before. Inversion has takes place; a reducing sugar (glucose) has been formed, as in the similar experiment with cane-sugar. This experiment is the basis of the commercial manufacture of glucose.

DEXTRINE, $C_6H_{10}O_5$.

As explained above, a number of compounds are included under this head. They are the first hydration products of starch. The commercial dextrins is prepared by heating starch to 150-160° with or without water; also by drying starch at 100° previously suspended in very dilute nitric acid; or by treatment with acids or

malt and subsequent precipitation with alcohol. The behavior of the several varieties of dextrine to iodine has been indicated above and will be demonstrated in connection with the work on saliva.

Unlike starch, dextrine is very readily soluble in water. The solution is not fermented by yeast, but must first be hydrated further to maltose. Test a 1% solution as follows:

1). To some of the solution add tannic acid--No precipitate; distinction from starch, gelatin, albumin.

2). To some of the solution add a drop or two of iodine solution. What is the result? And to what is it due?

3). To some of the solution add Fehling's solution and boil. Ordinary dextrine contains, more or less of reducing substances.

GLYCOGEN, $(C_6H_{10}O_5)_n$.

This carbohydrate was first discovered in the liver and has since been shown to be present, in greater or less amount, in all the tissues of the animal body. The amount of glycogen in the liver will vary according to the food. Ordibarily it constitutes from 1-4% but may, after a rich carbohydrate diet, amount to 12-16%. In fresh muscle it amounts to about 0.6% and disappears from the muscle as a result of work or starvation. It is present in Liebig's meat extract (1-15%). Although present in small amounts in normal blood, it is considerably increased after extirpation of the pancreas. It is present in larger amount in pus, and in leucocytes. Undoubtly it is a constituent of all living animal cells. It is abundant in embryonic tissue and the liver of a new-born dog has been found to contain as much as 11%. It is present in considerable quantity in molluses, notably in oysters. It has been found in certain plants, notably fungi, as truffles; also in mucor and in the yeast.

Glycogen is related to dextrine and to amylodextrin or soluble starch. It has the same precentage composition as starch or dextrine. The exact formulae is not known. It would seem that the multiple 'n' in the above formula is 6 although some place it at 10. Glycogen is derived form the food, more especially the carbohydrates. The excess of carbohydrates, whether starch or various sugars, is promptly stored up in the liver to be given off under the influence of ferments according to the need of the body. Exclusively proteid diet likewise gives rise to glycogen.

Various diastatic ferments such as are present in malt, saliva, pancreas, blood, liver, etc. invert glycogen. The change is similar to that which starch or soluble starch undergoes underlike conditions. That is to say, Erthyro dextrines, achroodextrine, iso-maltose, maltose, and eventually glucose form. On heating with water at high temperature, or with dilute acids, a similar hydration results. Owing to the action of these ferments of the body it follows that in dead liver or muscle the amount of glycogen rapidly decreases. and is replased by a dextrine body, or bymaltose or glucose.

The following table shows the relation of glycogen to sugar in a rabbit; liver at different periods after death.(Girard).

10 minutes.		24 hours.		48 hours.	
Sugar	Glycogen:	Sugar	Glycogen:	Sugar	Glycogen
0.75%	9.56%	3.58%	6.35%	3.85%	4.28%

The action of the diastatic ferments is most marked in neutral or very slightly acid solutions. A 1% solution of sodium carbonate inhibits the change and so does the acid solution of CO_2. It is possible that the carbonic acid prevents or retards the hydration of glycogen in the body. Glycogen is not affected by yeast.

Glycogen is an amorphous, white, tasteless powder which dissolves in warm water to form an opalescent liquid. The opalescence disappears on the addition of an acid or alkali. On the addition of iodine the solution becomes red or brown (Erythrodextrine). The color like that of starch iodine disappears on heating. The solutions are strongly dextro-rotatory. It is precipitated from impure solution by alcohol.

1). Isolation of Glycogen.--The following method gives the best results. It may be applied to 50g of perfectly fresh liver, or to 1/2 pint of oysters. The material is cut up as fine as possible. If liver is used it can be put through a sausage machine. To the material then add 10 parts of boiling water slightly acidulated with acetic acid. Strain the opalescent liquid through muslin. This liquid contains besides glycogen some proteids and gelatin. To remove the latter first concentrate to a small volume, then add alternately a few drops of HCl and of potassium mercuric iodide till a precipitate ceases to form. Finally filter off a little of the liquid and test it with acid and reagent to make sure that all the proteids are precipitated. If this is the case strain the liquid through muslin, then filter through paper and to the filtrate add two volumes of alcohol and stir thoroughly. Allow the glycogen to settle, then filter off, wash with dilute alcohol (2 parts alcohol to 1 part water). Finally transfer to a beaker, cover with absolute alcohol and let stand an hour or more. Then filter off the glycogen fold the filter and gently squeeze off excess of alcohol, finally press between several layers of filterpaper till dry. Powder, if necessary.

The reagent employed above is prepared by mercuric iodide to a warmed 5% solution of KI till it ceases to dissolve. The liquid is then cooled and filtered.

With glycogen isolated as above make the following tests.

1). To some glycogen in a small beaker add 20-30cc of water and warm. The glycogen dissolves forming an opalescent liquid. Resemblance to soluble starch.

2). To a portion of the solution just obtained add a few drops iodine solution (in potassium iodine). A reddish brown color forms. Then heat the contents of the tube. The color disappears to reappear on cooling. Resemblance to Erythrodextrin and to starch iodine. The presence of pepton interfers.

3). Boil another portion of the glycogen solution with Fehling's solution. Note the result.

4). To some of the glycogen solution add a few drops of HCl and boil a few minutes. Then cool and neutralize, and test a portion with iodine; another portion with Fehling's solution. Compare with Exp. 2 and 3.

5). To some of the glycogen solution add about 1cc of saliva and mix. At the end of 10 minutes examine a portion with iodine; another portion with Fehling's solution. What is the result?

CELLULOSE, $(_6H_{10}O_5)_n$.

Cellulose, or wood-fiber, is present in all higher plants and as a rule in the lower plants including fungi and bacteria. It largely makes up the walls of the cell. Cellulose is probably formed by the protoplasm of the cell out of the carbohydrates that result from the assimilation of the carbonic acid of the air. The molecule of cellulose is probably much more complex than that of starch. Moreover it is probably that there are various distinct cellulose bodies. Tunicin or animal cellulose is found in some lower animals as the Tunicata, and is identical with plant cellulose and yields on decomposition dextrose. Cellulose has been reported in the lungs, blood and pus of tuberculous patients (Freund).

Cellulose is characterized by its difficult solubility. It is insoluble in water, alcohol, dilute acids or alkalis. It is soluble in an ammoniacal solution of copper oxide or Schweizer's reagent and from this solution it can be precipitated, unaltered, in an amorphous form by acids, alcohol or water. Cellulose is furthermore characterized by its reaction with iodine and concentrated sulphuric acid. Treated with concentrated sulphuric acid and with iodine it gives a blue color. This is due to a so called amyloid substance which , however, is not identical with the amyloid found in the animal body. Indeed tha latter is not a carbohydrate but probably a proteid. In place of H_2SO_4 zinc chloride can be used.

It does not melt on heating but turns brown and eventually decomposes yielding various products.some of which have considerable industrial importance . Thus, there is formed methyl alcohol (wood-spirit), acetic acid, (wood-vinegar) and creasote (wood-tar).

Concentrated sulphuric acid dissolves cellulose and if this solution is treated at once with water a gelatious precipitate of soluble cellulose or amyloid forms. If the acid is allowed to act longer, or the solution is heated, no precipitation takes place on dilution. When paper is rapidly immersed in concentrated sulphuric acid to which 1/4 its volume of water has been added, and when washed in water, amyloid which is first formed is precipitated on the paper. The result is the tough parchment paper.

When the solution of cellulose in sulphuric acid is allowed to stand for some time, then diluted with water and boiled glucose forms. Some kinds of cellulose yield mannose. Unlike starch boiling with dilute H_2SO_4 has but little affect.

With concentrated nitric acid, or a mixture of nitric and sulphuric (1-3) acids, it forms various so called nitro-celluloses. These compounds are made use of in several important preparations.

Thus, Collodium, which is used in surgery and in photography, is a mixture of tri- and tetra-nitro cellulose dissolved in ether. Gun-cotton or pyroxylin is a mixture of the tetra- and hexa-nitrate. Smokeless powder, which has revolutionized modern war-fare, may be pure gun-cotton, or gun-cotton mixed with nitrate of barium and potassium, or gun-cotton mixed with nitro-glycerin in different proportions (Nobelite, cordite explosive gelatin). Powders are also made out of nitro-phenol (picric acid) and out of nitro-naphthalene.

A mixture of nitro-cellulose and cellulose can be drawn out into long glistening threads resembling silk--(wood-silk). The cellulose which is the basis of ordinary paper is obtained from wood by heating with calcium sulphate under pressure.

Cellulose has been obtained in the shape of Sphaerochrystals, or minute needles. Cotton and linen threads and Swedish filter paper are practically pure cellulose. In the dry condition it is permanent but in the presence of water it readily undergoes under the influence of bacteria, fermentative decomposition giving rise to marsh gas. This bacterial decomposition takes place in the intestines and marsh gas, acetic and butyric acids are formed. The cellulose of the food increases the peristaltic action of the intestines and consequently considerable nitrogen may escape absorption.

1). Examine under the microscope and sketch, cotton, linen, silk and wool fibers also hair. The linen fibers are a hollow tube with a thick wall and hence retain their shape, whereas the cotton fibers have thin walls which readily collapse and produce the twisted character.

2). Tear up a little "washed" filter paper into small shreds (or use cotton) and warm with fresh Schwiezer's reagent. The cellulose dissolves. Acidulate the solution with acetic acid when it precipitates in an amorphous form. The Schweizer reagent is obtained by adding sodium hydrate to a solution of copper sulphate in the presence of NH_4Cl. The copper hydrate precipitate is filtered off, washed and dissolved in 20% ammonium hydrate.

3). Immerse some shreds of "Washed" filter paper, or cotton, in a strong solution of potassium hydrate (1-1). Allow the reagent to act for 10-15 minutes till the paper becomes gummy. Then transfer to a dish of water, and wash thoroughly, then acidulate with a little dilute hydrochloric acid and add some iodine solution. A blue color, due to amyloid, results.

4). To some cotton or shreds of paper add 5-10 cc of cold sulphuric acid. As soon as solution results take a portion of it cool and dilute with water. A gummy precipitate of amyloid forms. Add iodine solution, it colors blue

Allow the remainder of the acid solution to then stand for some time then dilute with water and boil for 1/2 hour; cool, neutralize with potassium hydrate and test with Fehling's solution for sugar.

5). Dilute some sulphuric acid with one-half its volume of water and cool the mixture. Then immerse, for a few seconds, an ordinary filter paper; remove at once and wash in tap-water. The tough parchment-paper results.

CHAPTER III.

P R O T E I D S .

1. EGG ALBUMIN.

Apply the following tests which are, more or less, general reactions for proteids to a 2% solution, unless otherwise indicated, of egg albumin. A white of an egg is carefully poured into an evaporating dish, then cut up with scissors and 20cc of the liquid is diluted to 1 liter 1-50 (2%). After thorough shaking in a cylinder the liquid is filtered and the clear filtrate employed for the tests. Observe the frothing of the liquid on shaking.

Dilute 2cc of the egg albumin to 10cc and shake thoroughly (1-5) 20%; also, dilute 2cc to 4cc and shake till thoroughly mixed (1-10) 10%.

COLOR REACTIONS OF PROTEIDS.

The following color tests (1-6) are general reactions for proteids.

1). Biuret test.--To the albumin solution (1-50) add an equal volume of strong sodium or potassium hydrate. Then heat to boiling and add 1-2 drops of very dilute $CuSO_4$ solution. The solution becomes colored, pink to violet, according to the amount of copper sulphate used. An excess of copper must be avoided. Salts of nickel give a similar reaction.

Repeat the test omitting the heat. What is the result?

All proteids give the biuret test, some more readily than others. The hydrated proteids, albumoses and peptons, give the test in the cold, Gelatin gives in the cold, a bluish violet color, not purple red as in the case of peptons.

The biuret reaction would indicate that proteids contain the biuret or urea group. Diamids, such as oxamid and its derivatives, however, give similar biuret reactions, and it is possible that such diamid groups are present in the proteid molecule. It is possible to remove the diamid group and the proteid that results no longer give the biuret reaction (Schiff).

2). Millon's reaction.--To some of the albumin solution (1-50) add a few drops of Millon's reagent. A white precipitate forms which on boiling for 2-3 minutes becomes colored red. The liquid may become likewise red.

This reaction is due to the aromatic nucleus contained in the proteid molecule. It is given by phenol, tyrosin, etc.

Millon's reagent is prepared by dissolving in the cold 1 part of mercury in 1 part by weight of concentrated HNO_3 (1.40). Gentle heat is finally applied and when all is dissolved 2 volumes of water

are added. The mixture is allowed to stand for some hours and the clear liquid is then decanted from any crystalline sediment that may be present.

3). Xanthoproteic reactions.--To some of the albumin solution (1-50) add an equal volume of conc. HNO_3 Then heat to boiling till the precipitate turns yellow or gives a yellow solution. Cool and add an excess of NH_4OH or NaOH. The color changes to an orange yellow.

This test can be always incidentally applied to the precipitate or liquid obtained in Heller's test, or in the nitric acid and heat test (I,).

4). Adamkiewicz's reaction.--To 2cc of concentrated H_2SSO_4 add about 4cc (2volumes) of glacial acetic acid and mix. To the mixture add 1 drop of dilute egg albumin. The liquid changes, slowly on standing, more rapidly when slightly warmed to a beautiful reddish violet color. The reaction is not given by gelatin or gelatin pepton.

The presence of water interfers with the reaction. It is therefore desirable to use the dry proteid or 1 drop of a concentrated solution. The spectrum of the solution resembles that of urobilin.

5). Liebermann's reaction.--To about 3cc of conc. HCl add 1-2 drops of undiluted egg albumin. Boil the liquid for several minutes. A pink to a violet color develpps. Too much water interfers with the reaction.

6). Heat some albumin with conc. H_2SO_4 and a little sugar. A red color results. Excess of sugar interfers by imparting a dark caromel color to the liquid.

The proteid molecule contains one or more aromatic groups. This is seen in the fact that on decomposition three distinct groups of aromatic bodies . . form. This we may have 1st) the oxy-phengl group represented in phenol and in tyrosin; 2nd) the phenyl group represented in phenylacetic acid; and 3rd) the indol group represented by indol and skatol. The Xanthoproteic reaction is due to the formation of intro-products and is also due to the presence of the 1st group. Millon's reaction is due to the presance of the 1st group of compounds. It is not given by the 3nd or 3rdgroups. The Adamkiewicz reaction is due to the 3rd group of products. On the other hand the Liebermann's reaction is apparently not due to the aromatic group.

PRECIPITATION REACTIONS OF PROTEIDS.

7). Take 4 test-tubes label and equip as follows: To tube 1 add 1-2cc of the undiluted egg albumin; to tube 2 add 5cc of the egg albumin, 1-5; to tube 3 add 5cc of the egg albumin,

and, to tube 4 add 5cc of the solution, 1-50. Immerse the 4 tubes in a boiling water-bath for 5-10 minutes, after which examine and note the results. Test the reaction of tubes 2,3,4. Tube 1 coagulates solid, whereas tubes 2,3,4 are more or less opalescent but far from coagulation. Dilution of egg albumin with water renders it non-coagulable by heat. Compare with test, Blood-serum IV, 4.

8). In each of 4 test-tubes place 5cc of the egg albumin solution (1-50). To tubes 1 and 2 add respectively 1cc and 0.2cc of a 10% NaCl solution. To the tubes 3 and 4 add respectively 1 and 5 drops of a 1% acetic acid solution (1cc of glacial acetic diluted to 100cc) To a fifth tube containing 5cc of egg albumin solution (1-10) add 1cc of a 10% NaCl solution. Immerse the 5 tubes in a boiling water-bath for about 5 minutes, then examine and note the results. Test the reactions of tubes 3 and 4. In experiment 7, above, tube 4, which can be considered as a control for this experiment, on exposure to 100° shows only a very slight opalescence. The addition of a small amount of NaCl increases the opalescence (tube2); the same amount of NaCl as in tube 1, added to a stronger solution of albumin (tube 5) brings on coagulation on heating; and a larger amount brings on partial coagulation on the walls of the tube (tube 1). Now add 1 or 2 drops of the 1% acetic acid to tubes 1, 2, 5 and to tube 4 add 1cc of 10% NaCl and heat again. Prompt and complete coagulation results. The liquid is clear. In tube 3 the addition of one drop of the diluted acid, thus changing the liquid to a very slight acid reaction, suffices to produce on heating a precipitate. A very slight excess of the acid (as in tube 4) prevents coagulation by heat. If NaCl however is added coagulation promptly results.

In attempting to remove albumin completely from a solution, as in the case of urine, it should be remembered that very dilute solutions must be barely acidulated with acetic acid. Furthermore, that the presence of NaCl favors coagulation on subsequent heating.

Albumin coagulates in a slightly acid or neutral solution, especially in the presence of a neutral salt or NaCl. Globulin requires a neutral salt to keep it in solution and this moreover favors coagulation on heating. Haemoglobin on heating decomposes into haematin and globin; the latter as just stated coagulates on heating in the presence of a neutral salt. Neucloalbumin is coagulated or thrown out of solution by acetic acid alone. The albumoses as will be seen later are precipitated by NaCl and the precipitate unlike albumin and globulin dissolves on heating. Peptons are not coagulated by heat

9).To about 5cc of the albumin solution (1-50) add an equal volume of concentrated HNO_3 so that the two liquids do not mix. This is done by allowing the acid to slowly run down the side of the inclined tube. A white cloud forms at the zone of contact of the two layers (Heller's test). Now mix the two liquids and gently warm. A flucculent precipitate separates. Now heat the mixture to boiling. In a short time the precipitate dissolves, acid albumin

forms, and the liquid is colored yellow. Cool the liquid and add an excess of NH_4OH. An orange yellow color results(Xanthoproteic reaction).

Egg albumin is therefore coagulated by HNO_3. The solution of this precipitate on boiling shows a distinction between this and the serum proteids.

, Mineral acids, such as HNO_3, coagulate albumin and globulin. The albumoses are precipitated by HNO_3 especially if NaCl is present, but the precipitate readily dissolves on the application of heat and reappears on cooling. Peptons are not precipitated by acids.

a). The test employed most often for the detection of albumin (and globulin) in the filtered urine is the coagulation or nitric acid and heat test. The reaction when properly carried out is exceedingly delicate. The best proceedure is as follows: To the urine add some concentrated HNO_3 so as to form two layers (see above). A precipitate or cloud indicates albumin. Now mix the two liquids and heat. A presistent flocculent precipitate is due to the albumin or globulin or both. Should it be necessary to decide whether this precipitate is due in part or whole to albumins, it can be done by saturating the urine with $MgSO_4$ according to directions given under Globulin Test 5.

If heat is applied direct to the urine a precipitate of phosphates may form. This, however, dissolves readily in HNO_3. If the urine is alkaline the HNO_3 should be added first to prevent formation of alkaline albuminate.

Apply the test as just given to some albuminous urine.

10). To about 5cc of the albumin solution (1-50) add 1-2 drops of strong acetic acid, then add 1-2 drops of potassium ferrocyanide. A voluminous precipitate forms.

This is a very delicate test for all proteids. It is not given, however, by peptons. The presence of NaCl favors the precipitation of the album oses. Moreover the albumone precipitate dissolves on heating and reappears on cooling.

This test and the nitric acid heat test, given above, are commonly employed for the detection of albumin in the urine.

If, in the case of urine, the amount of the precipitate is small and its nature doubtful it should be transferred to a filter and washed. The precipitate can be transferred by means of a glass rod to a test-tube and Millon's reagent added, If on heating a reddish coloration gorms it indicates ahe presence of a proteid. Another procedure is to add 1/2cc of the boiling Millon's reagent direct to the precipitate on the filter(Winternitz).

11). Strongly acidulate some of the albumin solution (1-50)

with HCl then add a few drops of phosphotungstic acid. A heavy white precipitate results. It is given by all proteids. Phosphomolybdic acid behaves in a similar manner.

12). Acidulate another portion as above and add a few drops of a solution of potassium mercuric iodide. Note the results. Why was this reagent used in the preparation of glycogen?

13). To a portion of the albumin solution (1-30) add 1-2 drops of tannic acid. What is the behavior of tannic acid to starch? To dextrin?

14). To another portion of the solution add a few drops of picric acid. A yellow voluminous precipitate forms. This reagent is used in Esbach's method for the estimation of albumin in urine.

The reagents employed in tests 10-14 inclusive are sometimes spoken of as alkaloidal reagents because of their reactions with the vegetable alkaloids and other bases. They are general reagents for proteids.

15). To 5cc of the albumin solution (1-50) add one drop of mercuric chloride. A heavy white cloud or precipitate results. Divide the cloudy liquid into two portions.

a). To one add an equal volume of a 10% solution of NaCl. The precipitate promptly dissolves even if mercury is in large excess.

b). To the other portion add two volumes of the diluted egg solution and mix. The precipitate dissolves if too much mercury has not been added.

16). To another small portion of the egg albumin solution add 1-2 drops of dilute lead acetate and note the result.

17). To a portion of the solution add 1-2 drops of silver nitrate. A voluminous white precipitate forms which on the addition of NH_4OH dissolves.

Experiments 4, 5, 6 are made with the salts of the heavy metals which precipitate most of the proteids. Why is the white of eggs administered in case of poisoning with corrosive sublimate or with salts of other heavy metals? Why should a stomach pump be subsequently used?

18). To about 3cc of the albumin solution (1-50) add 10-15cc of strong alcohol and mix. If no precipitate forms, but merely a cloudiness, then add 1/4-1/2cc of a 10% solution of NaCl. A voluminous white precipitate results.

Alcohol added in large excess (10 volumes or more) precipitates all proteids. The presence of NaCl favors the precipitation.

19). Place 10cc of egg albumin (1-50) solution in a small beaker or test-tube or pot. Add about 7g of powdered $(NH_4)_2SO_4$ and immerse in a water-bath at about 35° for half an hour. Stir frequently till the salt ceases to dissolve. Notice the heavy white precipitate that forms (albumin and globulin). When saturated transfer the contents to a dry filter. Test the filtrate:

a). By acetic acid and heat.
b). By the biuret test in the cold.

20). Place 10cc of the egg albumin solution (1-50) in a small beaker, as above, add about 12g of $MgSO_4$ and digest, with frequent stirring, at 35° for about half an hour. Observe that only a very slight cloud or precipitate forms (globulin). Filter through a dry filter and test the filtrate as in Exp. 19. What proteid is frequent in the filtrate? In the biuret test a large excess of NaOH should be added owing to the precipitate of $Mg(OH)_2$ that forms.

21). Determination of the coagulation point of albumin.--Place about 5cc of the undiluted agg albumin in a test-tube. Close the tube with a stopper through which passes a thermometer. The bulb of the thermometer should bearly touch the bottom of the tube and should be completely immersed in the albumin. Suspend the tube thus equipped in a large beaker of water. Fully two-thirds of the tube should be immersed. Heat gradually the water in the beaker and stir continnually by means of a glass rod bent at right angles. Note the temperature at which the albumin clouds. The albumin then becomes sticky : does not flow readily when inclined and finally becomes solid. Note the coagulating point of egg albumin.

22) .To 20cc of the 2% albumin solution add 2-3 drops of concentrated HCl and boil. No precipitate forms owing to the formation of an acid albumin.Cool the solution, a) to a portion add an excess of concentrated HCl a precipitate formsthat is difficultly soluble in excess. b)then in the remainder that is good place a litmus paper and add, drop by drop, very dilute NaOH. Mix the contents well after each addition of alkali. As soon as a precipitate or cloud forms note the reaction of the liquid. The precipitate of albuminate forms while the liquid is still acid. After the precipitate has formed add 2-3 drops more of the dilute NaOH. It dissolves at once to form an alkali albuminate.

23). To 10cc of the albumin solution add 1-2 drops of NaOH solution and warm gently for a few minutes. An alkali albuminate, forms. Raise the solution to boiling. It does not coagulate. Cool, add litmus paper and carefully neutralize, as above, with dilute HCl. What is the result? What is the effect of a slight excess of HCl?

Report the results obtained with egg albumin and with proteids subsequently to be studied in a tabular form such as the following:

	Egg albumin.	Serum albumin.	Serum globulin.	Albumose.	Peptone.	Gelatin.	
Biuret 1.							
Millon 2.							
Xanthoproteic 3.							
Adamkiewicz 4.							
Boiling							
Nitric acid 9.							
Acetic and ferrocyanide 10.							
Phosphotungstic acid 11.							
Pot. Mercuric iodide 12.							
Tannic acid 13							
Picric acid 14							
Mercuric chloride 15.							
Lead acetate 16.							
Silver nitrate 17.							
Alcohol 18.							
Ammonium sulphate 19.							
Magnesium sulphate 20.							

CHAPTER II.

SERUM ALBUMIN AND SERUM GLOBULIN.

Globulin.--is usually associated with albumin, though it may sometimes, as in the urine, occur alone. The tests given for albumin, as well as the general proteid reactions, are also given by globulin. For the separate recognition of albumin and globulin, when both are present in solution, it is necessary to resort to precipitation by either of the following methods:

1). Precipitation with $MgSO_4$.--To 10cc of blood-serum, in a small beaker or test-tube or pot, add 10cc of saturated $MgSO_4$ and 15g of powdered $MgSO_4$. Immerse the beaker or tube in a water-bath at a temperature of 30-35°. Stir frequently for 1/2-1 hour, until the $MgSO_4$ ceases to dissolve and the liquid is saturated. The globulin is thrown out of solution. Transfer the liquid and precipitate to a small filter. Save the filtrate (a) which contains albumin. When the liquid has drained through wash the residue 2-3

times with saturated $MgSO_4$. Finally spread out the filter on a flat surface, transfer the percipitate by means of a spatula to about 20cc of water. Globulin when pure does not dissolve in water but in this case, owing to the presence of salts, it dissolves.

Filter the solution and the clear filtrate (b) containing the globulin is reserved for experiment 3.

to

Apply the origional filtrate (a) which contains serum albumin the tests enumerated in the table. Note the results. Wherein does egg albumin differ from serum albumin? Boil a portion of the serum albumin solution to coagulate the albumin. Filter and apply the biuret test to the filtrate. What are the results?

2). Precipitation by semi-saturation with $(NH_4)_2SO_4$.--To 10cc of blood serum as above, add 10 cc of saturated $(NH_4)_2SO_4$. Immerse in a water-bath at 30-35° for about 1/2 hour and stir frequently. Then transfer the contents to a small filter. Save the filtrate (A) which conhains albumin. Wash the residue on the filter 2-3 times with semi-saturated $(NH_4)_2SO_4$. Finally spread out the filter on a flat surface, transfer the precipitate to about 20cc of water. The globulin precipitate dissolves for the reasons given above under 1.

Filter the solution and combine the clear filtrate (B) with the corresponding filter from experiment 1. The resulting solution is used for experiment 3.

The precipitate obtained by this method is larger than that obtained by the $MgSO_4$ method. The liquid filters much easier.

3). Separation of salts from globulin by dialysis.--Place the combined filtrates (B) in a dialyzer and dialyze against running water. Every day remove a few drops of the liquid from the dialyzer with a pipet and add to some dilute $BaCl_2$ solution. The dialysis should continue till all the sulphates are completely removed. This may require 3-5 days. In warm weather to prevent decomposition it is well to add a few drops of thymol.

When the sulphates have dialyzed out the globulin is thrown out of solution as a white granular precipitate. Now transfer the contents of the dialyzer to a small beaker. Pour into the dialyzer about 20cc of a 2% solution of NaCl and gently agitate to dissolve any precipitated globulin. Add this saline solution to the contents of the beaker and stir till the globulin dissolves. Finally allow the liquid to stand for a while then filter. The clear filtrate now contains pure globulin. Observe the frothing of the liquid on shaking.

To this solution of globulin apply the tests enumerated in the table on page 23. Make careful records of the results obtained. The presence of NaCl will interfer with the tests 16-17.

Boil a portion of the globulin solution to coagulate the globulin To the filtrate apply the biuret test.

4). To 10cc of blood-serum add an equal volume of saturated $(NH_4)_2SO_4$. Then add 8g of powdered $(NH_4)_2SO_4$ and immerse in a water-bath at 30-35°, stirring frequently, for about 1/2 hour. The liquod becomes saturated with $(NH_4)_2SO_4$ and a precipitate forms. Finally transfer the filter. Notice the perfect clearness of the filtrate.

Test a portion of the filtrate by boiling; another portion with tannic acid.
To another portion apply the biuret test in the cold.

The absence of proteids in the filtrate demonstrates that albumin and globulin and completely precipitated on saturation with $(NH_4)_2SO_4$. The absence of a biuret reaction indicates the absence of a pepton .

5). Detection of globulin in the urine.--As indicated under albumin (Experiment 9 a, page 20) the ordinary tests for albumin are also given by globulin. In order to ascertain positively which of the two, or if both are present it is necessary to resort to the saturation method with $MgSO_4$. For this purpose 100cc of the urine can be taken and neutralized. 120g of powdered $MgSO_4$ are then added and the liquid kept at 30-35°, with frequent stirring, till the $MgSO_4$ ceases to dissolve. If globulin is present a precipitate will form. This can be removed by filtration washed with saturated $MgSO_4$ solution, and finally dissolved in water (See page, Globulin II. 1.) This solution of the $MgSO_4$ precipitate can now be tested. It coagulates on heating especially if slightly acidffied with acetic acid. It gives the nitric acid and heat tests, also the biuret reaction.

The filtrate from the $MgSO_4$ precipitate contains albumin if any is present. The tests just given applied to this filtrate, if positive, prove the presence of albumin.

A L B U M O S E III.

This compound or rather group of compounds can be readily prepared from White's or Schuchardt's commercial pepton since this consists largely of albumoses. Albumoses are precipitated by saturation with $(NH_4)_2SO_4$ or with NaCl in an acid solution.

A 20% solution of the commercial pepton is used. The powder readily dissolves especially if the liquid is warmed and thoroughly stirred.

1). Place some of the solution in a test-tube and heat to boiling. The liquid does not coagulate thus indicating the absence of albumin and globulin.

2). To 10cc of the solution add 10cc of saturated $(NH_4)_2SO_4$ solution and about 2g of powdered $(NH_4)_2SO_4$. Saturate the liquid in a water-bath at about 30-35° according to the directions given in experiment II, 4. Notice the sticky precipitate that adheres to the rod and to the sides of the beaker or tube. Since albumin and globulin are absent (Exp? 1.) the precipitate that forms consists of albumoses. Transfer the precipitate to a filter and wash with about 10cc of saturated $(NH_4)_2SO_4$.

Save the filtrate (A) for subsequent tests for pepton.

By means of a glass rod gather the sticky albumose predipitate and transfer it to about 20cc of water in a test-tube. While stirring, heat the liquid carefully and the albumose dissolves completely.

With this aqueous solution of pure albumose make the following tests, employing small quantities of the liquid about 1cc.

a.) Heat a portion to boiling. It does not coagulate.

b.) To a portion add NHO_3, drop by drop. A slight precipitate may form which dissolves and gives a yellow solution. If there is no permanent precipitate add some saturated NaCl, drop by drop, till a precipitate does form. Now heat gently the contents of the tube. The precipitate dissolves and on cooling reappears. This reaction is characteristic for the albumoses.

In the absence of NaCl some of the albumoses, especially dentero albumose, do not give a precipitate with NHO_3 An excess of NaCl, however, should be avoided since in that case the albumose precipitate does not dissolve completely on heating.

c.) To a portion of the solution add a few drops of acetic acid (1-10) and 2-3 drops of potassium ferrocynide. If no precipitate forms add NaCl according to directions given above under c.). A precipitate then does form and on heating gently it dissolves. On cooling the solution it reappears.

This reaction, like the predeeding, is also characteristic if albumoses. A certain amount of NaCl is necessary as in the NHO_3 test.

d.) To about 1cc of the solution add 1-2 drops of dilute acetic acid and about 5cc of a saturated NaCl solution. A precipitate or cloudiness results. On heating this disappears, to reappear on cooling.

To the remainder of the solution of albumose apply the tests given in the table (page 23) and note the result. In which of these reactions will the presence of chlorids and of ammonium salts interfer?

Apply the biuret test without the aid of heat. The hydration proteids give this reaction readily in the cold.

3). Detection of albumose in the urine.

The reactions given above under 2, especially b, c, and d, are characteristic of albumoses. To detect albumos in the urine, or in other liquids, it is necessary first to remove the albumin and globulin. This can be readily done by acidifying very little with acetic acid and applying heat. The albumin and globulin coagulate. To the filtrate the biuret test can be applied. If the result is negative it indicates the absence of albumoses and also of pepton. If, however, the result is positive it is due either to albumoses or to pepton, or to both. The tests given above under 2b, c, and d can now be applied and if positive, the presence of albumose is demonstrated. If these tests fail the positive biuret reaction is due to pepton.

PEPTON, IV.

Pepton is not precipitated by $(NH_4)_2SO_4$. The filtrate (A) obtained in experiment 2 under albumose therefore contains pepton if it be present.

To this filtrate apply the biuret test in the cold. A positive reaction is due to pepton. As indicated before the hydrated proteids, as a rule, require heat,

To obtain a pure solution of the pepton it would be necessary to resort to dialysis, or to treatment with baryta on a water-bath to remove the $(NH_4)_2SO_4$.

To the origional filtrate containing pepton apply the tests given in the table on page 23 and note the results. With which of these reactions will the $(NH_4)_2SO_4$ present interfer?

Detection of peptons.

To about 500cc of the urine, or to an aqueous extract of the tissue to be examined, made at about 40°, add just enough lead acetate to give a strong presipitate and filter. This removes mucin. Test the filtrate for albumin and if present remove in the following manner: Add a little sodium acetate and then concentrated ferric chloride till the mixture is blood red in color. Then neutralize with potassium hydrate (or leave slightly acid), boil, cool and filter. The filtrate should give no precipitate with acetic acid and potassium ferrocyanich (absence of iron and of albumin). If it is perfectly free from albumin make the following tests:

1.) Add acetic acid and phosphotungstic acid--a cloudiness forms on standing if pepton is present.

2.) If pepton is indicated by the above trial it can be isolated by the following method: Add 0.1 volume of concentrated hydrochloric acid and then phosphotungstic acid also acidulate with hydrochloric acid, as long as a precipitate continues to form. Filter at once and wash with dilute sulphuric acid (3 to 5cc in 100cc water), till the filtrate is colorless. While the precipitate is still moist mix it with an excess of powdered barium hydrate, add a little water, gently warm for a short time and filter. To the filtrate which contains pepton apply the biuret test. (Hofmeister's method).

This method does not indicate true peptones only, but also albumose.

Another method for the detection of pepton is based upon its behavior to $(NH)SO_4$. The method as employed by Devoto is as follows: To 200-250cc of the urine add 80% by weight of $(NH_4)_2SO_4$. This is added to urine even if albumin and globulin are absent in order to remove neucloalbumin. Warm the mixture on the water-bath till the salt didsolves. This will occur in 10-15 minutes.. Now place the beaker in a steam sterilizer for 30-40 minutes or longer. The albumin coagulates completely irrespective of the reaction of the fluid. The mixture is allowed to cool, then filtered The filtrate can be tested by the biuret reaction. If positive pepton is present. It can further be precipitated with tannic acid.

The residue on the filter can be washed with hot water will the filtrate ceases to give a test for $BaSO_4$. If the filter has previously been dried and weighed, and is now again dried and weighdd the difference is due to the albumin and globulin. (See extimation of albumin and globulin.)

The first portions of the hot wash-water are collected combined and tested by the biuret reaction. If positive it is ordinarily said to be due to pepton (Devoto, Jakash.)

The Hofmeister method will often give positive results where Devoto's method fails.

In reality the reaction in that case is due to albumoses. The true pepton which would be present in the filtrate from the cold saturated solution seems to be very rare in mine.

As used in a clinical way the term "pepton" includes pepton and albumoses. Such pepton may be present, though not always, in the blood of the leukaemics during life. The blood obtained from deceased leukaemics, especially if decomposition has set in is rich in such pepton. The normal liver does not contain pepton. whereas the spleen does. The liver and spleen of leukaemics is rich in such pepton.

Another process for the detection of true pepton is as follows:

Saturate the solution at the boiling point with ammonium sulphate and filter while boiling hot. Allow the filtrate to cool, decant the liquid from the crystals which separate, dilute strongly and precipitate the pepton by cautious addition of tannic acid. Let stand for 24 hours then filter. Boil the precipitate for a few minutes with baryta water filter and from the filtrate remove the excess of barium by passing carbonic acid. Filter off the barium carbonate and test the filtrate for biuret.

GELATIN, V.

To study the reactions of gelatin a 2% solution of the best French gelatin (silver) is employed.

1). Shake up some of the solution. Notice the foaming of the liquid.

2). To a portion of the solution add some bromine water. An abundant, yellow, sticky precipitate forms.

3). In each of two test-tubes add 1-2 drops of saturated $HgCl_2$ soluyion. To tube 1 add about 5cc of the gelatin solution. To tube 2 add an equal volume of water. Then add to each tube some H_2S-water and heat. Tube one is dark yellow but contains no precipitate, whereas tube 2 has a blackish precipitate of HgS and the liquid is clear. Gelatin prevents the precipitation of many otherwise insoluble, compounds.

4). To the gelatin solution apply the several tests given in the table on page 23. Tabulate the results, and carefully note the differences.

Observe that the heavy metald do not precipitate gelatin, whereas the other proteids are precipitated. Also, that gelatin is not precipitated by ferrocyanide even in the presence of NaCl and in this respect it resembles pepton.

The Xanthoproteid reaction is weak owing to the absence of the phenol group, C_6H_5OH. The biuret reaction applied to the cold solution of gelatin gives a bluish violet color, whereas pepton gives a purple red. Millon's reagent gives a white precipitate thich on heating becomes red and the liquid becomes pink. It is probably that aal the other reactions are not strictly due to the gelatin but to a mixture of some pepton or albumose.

CHAPTER IV.

SALIVA.

Saliva is a mixture of the secretions of the parotid, submaxillary, and sub-lingual glands. The reaction of mixed saliva is usually alkaline, but may on fasting also during the night toward morning, and 2-3hours after meals, or after much talking, become acid. It also becomes acid on standing a few hours. (Repin'. It is more or less opalescent and viscid and foams readily. The character of the saliva will vary according to which gland furnishes the most of the secretion. The protaid gland yields a fluid secretion whereas the submaxillary and lingual glands yield slimy secretions. In febrile diseases the secretion of saliva may be diminished or wholly suppressed, and hence dryness of the mouth and throat, as well as altered taste. A decrease is also observed in diabetes, in severe diarrhoeas, as in cholera. The administration of potassium iodide or of mercury produces an increased flow, or salivation, and the composition of the saliva itself becomes altered. Albumin becomes present and the amount of salts in solution is increased. An increased flow of saliva (ptyalism) is also brought about by irritant poisons such as acids and alkalis; also by certain foods, lemon, etc., and occurs also in some diseases, especially in inflammatory conditions of the mouth, tonsil, and palate.

In icteric conditions the saliva does not contain bile constituents. In diabetes it does not contain sugar. In the latter case, however, the action may be acid because of lactic acid. In nephritis, urea may be present in the saliva, and uric acid has been found in uraemic conditions. Leucin has been found in the saliva of a hysteric case.

Salivary calculi which are occasionally deposited in the salivary ducts consist chiefly of calcium carbonate and phosphate with organic matter. The tartar deposited on teeth has essentially the same composition, the phosphates however predominate. These calcium salts are held in solution in the saliva by carbonic acid. On exposure to the air this passes off and the salts are deposited.

The specific gravity of the mixed saliva varies from 1.002 to 1.008 and contains 1/2-1% of solids which consist of albumin, mucin, ptyalin, traces of urea and other nitrogen compounds and mineral constituents. The amount of saliva secreted in the course of 24 hours is 1400-1500 cc. The flow is increased after meals and by pilocarpin. Atropin diminishes salivary secretion.

The chemical examination of saliva has at present but little clinical significance. Physiologically, however, the composition and action of saliva is of the greatest importance. The ferment or enzyme present in the saliva is known as ptyalin and possesses a diastatic or amylolytic action. That is, converts starch into dextrin, then into iso-maltose and maltose. Eventually glucose forms probably however the result of the action of an inverting ferment. Ptyalin is not present in the saliva of all animals. The parotid saliva of new-born contains ptyalin, whereas the submaxillary saliva does not contain it for several months. In the saliva of some animals as horse the ferment is not present free but as a zymogen from which it readily forms in mastication. This as well as the other enzyme, is dragged down mechanically by a precipitate

of calcium phosphate and this fact is utilized to obtain the ferment in a comparatively free state.

Although ptyalin resemblesin its action the diastese of malt, it is different. This is seen in the fact that the former acts best at 40°, the latter at 50-60°. The amount of ptyalin present in the saliva is subject to variation. HCl not only prevents the action but it also destroys the ferment. The action of the ptyalin is mst marked in neutralized or very faintly acid saliva.

A microscopic examination of the saliva will always show epithelial cells from the mouth and tongue, also salivary and mucous corpuscles. Bacteria are always numerous, and certain species as the leptothrix, spirillum, and spirochaete are almost invariably present. Among the pathogenic forms found in the mouth in health or in disease may be mentioned the bacilli of diphtheria, tuberculosis and tetanus, Fraenkel's diplococcus, the micrococcus tetragenus and the pus-producing staphylococci and streptococci, the fungus of thrush and of active mycosis. Blood or pus cells may be present in the saliva in inflammatory suppurative conditions of the mouth, gums, etc.

Rub the tongue thoroughly over the inside of the mouth, teeth and gums, collect the saliva and examine under the microscope for epithelial cells, salivary corpuscles, etc.

The saliva necessary for the following experiments can be readily obtained by chewing a piece of pure paraffin. Commercial gum must not be used inasmuch as it contains sugar. Collect about 100 cc. of saliva.

1). Test the reaction of the mixed salivawith litmus paper. What is it?

2). Nearly fill a 50 cc. graduate with saliva. If there is any foam on the surface remove it with a piece of filter paper. Then immerse an urinometer and note the specific gravity of mixed saliva. What is the reading if immersed in pure water?

3). To about 5 cc. of saliva add a few drops of acetic acid (1-10) and gently agitate. A flocculent precipitate of mucous forms.

4). To some saliva apply the biuret test(Exp. p.). The result is due to mucin.

5). To some saliva add a drop of nitric acid and boil. Is albumin present in saliva?

6). To the contents of the tube from the preceding experiment add NH_4OH. An orange yellow solution forms xanthoproteic reaction.

7). To some saliva add a few drops of Millon's reagent. A heavy yellowish precipitate forms which on boiling becomes reddish. This is due to mucin.

8). To some of the saliva add a drop of dilute HCl, then, drop by drop, dilute ferric chloride till a red coloration results. This is due to the formation of ferric sulphocyanide. The reaction is more distinct kf after the addition of HCl the liquid is filtered and the ferric chloride is added to the filtrate.

9). To another portion of saliva add a little iodic acid and some starch solution. Iodine is liberated and colors the starch blue. This is due to a sulphocyanide. Explain the reaction.

10). To some saliva add a few drops of dilute H_2SO_4, mix; then add a few drops of a colorless solution of potassium iodide and finally a few drops of starch solution. Iodine is liberated and colors the starch blue. This is due to nitrous acid. Explain .

11). To some saliva add a ~~drop or two of dilute~~ HCl, then ~~2-3 drops~~

of a saturated sulphanilic acid solution and mix. Now add a few drops of naphthylamine hydrochloride. A pink or red solution indicates the presence of nitrous acid. This test is employed in testing for nitrates in water analysis.

12). Take a small dose of potassium iodide, rinse out the mouth thoroughly with water and test some of the saliva, at once for KI. This is done by adding to some of the saliva a little chlorine-water and then shaking with carbon bi-sulphide. A pink coloration of the latter indicates iodine. Iodine should be absent from the saliva after rinsing. After that collect a little of the salive every 10 minutes and test for iodine as above. How soon does KI appear in the salivaafter being taken into the stomach?

13. Separation of Mucin.-- Pour 10 cc. of the saliva slowly and with constant stirring, into 50 cc. of absolute alcohol. A fibrinous light precipitate forms. Allow to settle over night in a covered beaker. Then filter, wash the precipitate on the filter twice with alcohol, then with ether. Spread out the filter to dry and finally with a spatula remove the white chalky powder of mucin.

a. To a little of the powdered mucin in a tube add some water. It swells up but does not pass into solution. Then add a drop or two of KOH when it dissolves forming a milky solution. To this solution now apply the biuret test. What is the result?

b. Place the remainder of the power in a tube and add dilute HCl (1-3) and boil for some minutes. Transfer a portion to another tube, cool, render alkaline with KOH and boil with Fehling solution. The formation of red cuprous oxide indicates the presence of a reducing substance. If this test is not given, boil again, ~~and~~ the remaining original liquid and again test a portion as above.

Mucin is a complex proteid substance and on decomposition, as above, it yields a reducing compound which, however, is not sugar. What other substances on heating with an acid yield reducing substances

14). Action of Ptyalin.-- Prepare a starch solution according to the directions given under starch. Into each of the eight tubes place about 3 cc. of Fehling's solution. Into each of other set of 8 tubes place 1-2 drops of dilute iodine solution.

a. To 30 cc. of the salt solution in a graduate 6 drops of saliva are added at once and mixed thoroughly. Immediately after mixing pour 2-3 cc. of the mixture into a tube containing Fehling solution, and also into a tube containing the iodine. The latter colors deep blue--due to starch. Boil the tube with Fehling solution. No reaction should take place--absence of sugar.

At intervals of two minutes apply the test with Fehling solution and with iodine to the mixture in the manner just given. Tabulate your results, noting the time when sugar appears in the mixture; when erythro-dextrin and achroo-dextrin appear. The time of appearance of the latter is spoken of as the achromic point. When this is reached boil some of the starch mixture with Barfoed's reagent. What is the result? What does this indicate?

At the conclusion of this test add to each of the iodine tubes 5 cc. of water. The characteristic color of the starch and the several dextrines will be more apparent. Complete conversion should take place in about 15 minutes. If it does not repeat the experiment using a larger amount of saliva.

b. To ~~10 cc. of starch solution add 5 cc. of saliva,~~

mix and make tests as rapidly as possible with iodine and with Fehling solution. What is the result?

c). Boil 5 cc. of saliva in a tube for 1-2 minutes, then add 10 cc. of the starch solution and mix. Immediately test a portion as above and also at the end of 15 minutes. What is the result? What is the action of heat on ptyalin?

d). To 10 cc. of starch solution add 0.2 cc. of a 1% acetic acid solution, mix and then add 2 drops of saliva. Test immediately with iodine and with Fehling solution, and also at the end of 5, 10, and 15 minutes. The mixture contains about 0.02% acetic acid. What is the effect of this amount of acetic acid on the rate of inversion?

e). To 10 cc. of the starch solution add 0.6 cc. of a dilute HCl(0.3%). The latter is prepared by adding 10 cc. of the concentrated acid to one litre of water. Mix and then add 2 drops of saliva. And again mix thoroughly. This mixture now contains about 0.02% HCl, about the same degree of acidity as in the preceding experiment, and about .1 of that of the gastric juice. Test the mixture at once with iodine and with Fehlingsolution, and also at the end of 5, 10, and 15 minutes. Note carefully the result. How doesthe action of HCl compare with that of acetic acid.

CHAPTER V.

G A S T R I C J U I C E.

I. RECOGNITION OF FREE HYDROCHLORIC ACID.-

Three solutions of dilute HCl labelled 1, 2 and 3 will be found on the side-table. Solution 1 approximates in strength that found in the gastric juice. It is prepared by diluting 6cc of HCl (1.19 specific gravity) to one liter (=0.25%). Solution 2 is prepared by diluting 200cc of solution 1 to one liter (=0.05%). Solution 3 is prepared by diluting 200cc of solution 2 to one liter (=0.01%)

A 2% pepton and a 1% lactic acid solution will also be found on the side table. If the pepton solution is slightly alkaline it should be faintly acidified with acetic or lactic acid.

The following tests are given in the order of their delicacy.

1). Di-menthylamidoazobenzol.--This reagent is used in a 0.5% alcoholic aolution. Add 3-4 drops of the reagent to some of the solution to be examined. If a pink red color forms a free mineral acid is present. In the case of gastric juice it is HCl. A yellow color indicates an absence of HCl. Certain substances juch as pepton and organic acids tend to interfer in this as well a as in the subsequent tests.

Note the results obtained with solutions 1, 2 and 3 in the first column. Then mix the same amount of these solutions with an equal volume of 2% pepton and to this mixture apply the test and note the results in the second column. In the same way make a mixture of the three solutions with an equal volume of a 1% solution of lactic acid, test and note the results.

	aa 2% pepton.	aa 1% lactic acid.
1cc of Solution 1.		
1cc " " 2.		
1cc " " 3.		
Limit of delicacy.		

Apply the test to the solution of pepton, also tbethe solution of lactic acid. Report the results.

2). Günzburgés reaction of the phloglucin-vanillin test.--
The reagent is prepared by dissolving 1g of vanillin and 2g of phloroglucin in 100cc of alcohol.

Place the solution to be tested in an evaporating dish add 2-3 drops of the reagent and carefully evaporate over a small flame to dryness. A purple or pinkish-red color indicates free HCl. This has long been considered the most delicate test for free HCl.

Apply this test to solutions 1, 2 and 3 and to mixtures as indicated in the table given in experiment 1. Carefully note the limit of delicacy of the reaction under the several conditions. Tabulate results as above.

3). Boas' reagent.--This is prepared by dissolving 10g of resorcin, 3g of canesugar and 3cc of alcohol in 100cc of water. Place in an evaporating dish the solution to be tested, add 2-3 drops of the reagent and evaporate over a small flame to dryness. If a free mineral acid is present a rose or pink-red color develops and gradually fades on cooling.

Apply this test to solutions 1, 2 and 3 and to mixtures as indicated in the table given in experiment 1. Note the limit of delicacy and tabulate the results.

4). Tropaeolin OO.-- A solution of this reagent is prepared by dissolving 0.25 g of the reagent in 1000cc of water. Instead of the solution tropaeolin papers may be employed. They are , however, not so reliable as the solution since with distilled water they sometimes give a pink color. To some of the acid solution add a drop of the reagent (or immerse a strip of the tropaeolin paper). A pink color is due to free mineral acid. If the solutin is evaporated carefully to dryness a bluish residue remains.

Apply this test to the solutions as given in experiment 1 and tabulate the results.

5). Congo red papers.--The color of these papers is changed on contact with mineral acids to a deep blue, whereas organic acids yield a violet. Immerse a strip of the paper in 1cc of the solutions 1, 2 and 3, also lactic acid and distilled water and report the results and the delicacy.

6). Benzopurpurin 6B papers.-- These papers are turned to an intense dark brown color by mineral acids. With strips of this paper make similar tests as those given in experiment 5 and report the results.

7). Methyl violet.-- A solution of this reagent is prepared by dissolving 0.5g in 1000cc of water. To the solution to be tested add 1-2 drops of the reagent. Free hCl gives a copper-blue color. Organic acids yield a violet blue. Apply this test, first to some distilled water, and note the color. Finally apply the test to the solutions 1, 2 and 3 and compare the results. Also test pepton and lactic acid mixtures and tabulate the results as under experiment 1.

II. DETECTION OF LACTIC ACID.

Uffelmann's test.--The reagent is prepared by adding a drop of dilute ferric chloride to 10cc of a 2.5% carbolic acid solution. The liquid is colored blue. This color is completely discharged by mineral acids leaving a colorless solution, whereas organic acids discharge the color and leave a straw yellow solution. A 1% lactic acid solution os used.

1). In each of 3 test-tubes place 5cc of the reagent then add to each about 1/2 cc of the lactic acid solution. The blue is replaced by a straw yellow color.

To each of these tubes now add respectively an equal volume of the HCl solutions 1, 2 and 3. Note the interference, if any, in the lactic acid reaction by the presence of free HCl.

2). In each of the 3 tubes place 5cc of the reagent, then add respectively an equal volume of the HCl solutions 1, 2 and 3. Compare the results with that obtained above with lactic acid.

3). In each of 6 tubes place 5cc of an almost coloress solution of $FeCl_3$. To tube 1 add 1cc of the HCl solution 1. To tube 2 add 1cc of the lactic acid solution. To tube 3 add 1cc of the 2% pepton solution. To tube 4 add 1cc of alcohol. To tube 5 add 1cc of a 4% solution of canesugar. Tube 6 remains blank and serves for a companion. Carefully note the results.

It is evident from the above experiments that this test for lactic acid is not characteristic. In the first place free HCl if present in sufficient amount may interfere; and secondly, a similar test is given by a number of substances which may at times be present in the stomach contents. In order to obtain a positive test for lactic acid it is necessary to isolate the lactic acid from the liquid by extraction with ether. The liquid must be extracted several times with ether. The ether is then distilled off, the residue dissolved in water and tested as above.

III. PEPTIC DIGESTION.

The following solutions will be found on the side-table.

1). A 0.25% solution of HCl. This solution is the same as Solution 1. used in connection with the tests for free HCl. It corresponds to the normal acidity of the gastric juice.

2). A solution of pepsin in water. This is prepared by dissolving 1g of pepsin in 1000cc of water.

3). A pepsin-hydrochloric acid solution. This is prepared by dissolving 1g of pepsin in 1 liter of solution 1.

Label 6 tubes and equip as follows:

1). Place in tubes 1 and 2 20cc of solution 3 and about 2g of fresh washed fibrin. Too much fibrin should be avoided.

Place in tube 3 10cc of solution 3. Immerse in boiling water for about 2-3 minutes; then cool to 40°, and add 2g of fibrin.

Place in tube 4, 10cc of solution 1 and about 2g of fibrin.

Place in tube 5, 10cc of solution 2 and about 2g of fibrin.

Place in tube 6, 2.5cc of solution 3 and 7.5cc of solution 1. Then add about 2g of fibrin.

The tubes thus prepared are placed in an incubator at 40° or immersed in a water-bath having that temperature. At the end of 15 minutes the tubes are taken out and examined. Observe that in all the tubes, except tube 5, the fibrin has swelled up so that the contents of the tube are solid. Return the tubes to the incubator and examine at the end of every hour for the next three hours. Observe the change that takes place in tubes 1 and 2 and compare carefully with tubes 3, 4 and 5 and with tube 6.

The tubes remain in the incubator till next day. If, however, tube 1 is completely digested in 2-3 hours it should be treated at once according to experiment 2. Then carefully examine and note the condition of each tube. In tubes 1 and 2 and possibly in tube 6 the fibrin has disappeared. A finely granular, whitish or brownish sediment is left. What is it ? Tubes 3 and 4 are about alike. The fibrin is gradually being dissolved by the dilute acid. The pepsin added to tube 3 evidently has been destroyed by boiling. No change in tube 5.

Return tubes 2, 3 and 4 to the incubator and keep there for 3-4 days longer.

2). Filter the contents of tube 1.

a). To 10cc of the filtrate, in a small beaker, or in a wide test-tube or pot, add 8g of powdered $(NH_4)_2SO_4$. Immerse for 1/2-1 hour in a water-bath at a temperature of 30-35°. Stir with a rod frequently to bring the salt into solution. When the salt ceases to dissolve, iewhen the liquid is saturated the albumose present will be thrown out of solution as coarse floccules which rise to the surface forming a sticky or slimy layer. Transfer the liquid to a filter previously moistened with a little saturated $(NH_4)_2SO_4$ solution.

Wash the residue with 10cc of saturated $(NH4)_2SO_4$ solution.

a'). The clear $(NH_4)_2SO_4$ filtrate contains pepton. Test this solution as follows:

1). To a liter of the liquid add an equal volume of strong NaOH, then 1-2 drops of very dilute $CuSO_4$ solution. A pink color results. The biuret test is given in the cold by the hydrated proteids.

2). To another portion of the filtrate add 1-2 drops of a fresh tannic acid solution. Avoid an excess of reagent. A heavy white precipitates forms.

3). To a portion add one to two drops of dilute acetic acid, then a drop or two of potassium ferrocyanide. What does the absence of a precipitate mean?

b'). The $(NH_4)_2SO_4$ precipitate left on the filter is albumose. Transfer to a tube, add distilled water, warm gently and stir with a rod till dissolved. Test this solution as follows:

1). Boil the solution. Absence of coagulation shows absence of albumen and globulin.

2). To a portion apply the NHO_3 and heat test for albumen.

3). To another portion apply the acetic acid and potassium ferrocyanide test for albumene.

c'). With the remainder of the filtrate from tube 1 make the following tests:

1). Heat a portion to boiling. What does the absence of coagulation mean?

2). To a little of the liquid (1cc) apply the biuret test as given above under a'-1.

Exactly neutralize the remainder of the solution with dilute NaOH and test for albumoses as follows:

3). To a portion add 1-2 drops of dilute acetic acid and a drop or two of ferrocyanide solution. If no precipitate forms add some NaCl solution according to directions given test.

4). To another portion apply the NHO_3 and heat test for albumose.

5). To the yellowish liquid obtained in tube 2 add an excess of NH_4OH. An orange yellow color results--the Xanthoproteic reaction.

6). To another portion of the liquid add 1-2 drops of fresh tannic acid solution. Heavy white precipitate.

3). After tube 2 has been kept for 3-5 days at 40°, filter the contents. Saturate 10cc of the liquid with $(NH_4)_2SO_4$ according to the directions given anove. The liquid is cloudy but very little albumose is precipitated. Why?

Filter the saturated liquid and to a portion of the filtrate apply the biuret test as given above under a'-1.

4). The fibrin in tubes 3 and 4, in a few days at 40°, is completely dissolved by the acid present. When this occure unite the contents of the two tubes and filter. Exactly neutralize the filtrate according to directions given in test 22, a heavy white precipitate shows the presence of acid albumen or as it is sometimes called syntonm. Pepton may also form but will remain in solution.

IV. EXAMINATION OF STOMACH CONTENTS.

1). The stomach and contents of a recently fed rabbit (or larger animal) are cut up, diluted with about 500cc of water and placed at 40° for about 1 hour. The mixture is then filtered through muslin. This dilute gastric juice is used for the following experiments:

1). Test the reaction with litmus paper. It is distinctively acid.

2). Test portions of the liquid for free HCl according to I, 1&2.

3). Test a portion for lactic acid according to II, 1.

4). To 10cc of the solution add a shred of fibrin or a flake of coagulated egg albumin. Set aside at 40° for 2-3 hours. If not dissolved let the tube remain at this temperature over night. Then filter and to the filtrate apply the biuret test in the cold. III, 2a.

5). Apply the biuret test direct to a portion of the dilute gastric juice and compare the intensity of the reaction with that obtained in 4.

6). In each of 3 test-tubes place 10cc of fresh milk. To tube 1 add 2cc of the solution, preciously carefully neutralized. To tube 2 add one drop of commercial rennet solution. Tube serves as a control. Set the tubes aside at 40° for 1 hour then examine.

2). Test for pepsin.--The following test is applicable to vomited matter, or the liquid obtained from a stomach. Dilute 20ccc of the liquid, if neccessary, and filter, To one half of the filtrate in a test-tube add a few shreds of washed fibrin, or a flake of coagulated egg albumin. Set aside at 40° for 1/2-1 hour. The fibrin should dissolve, the egg albumin requires more time. If no digestion takes place it may be due to the absence of HCl, or of pepsin or of both.

To the other half of the filtrate add an equal volume of 0.5% HCl. This is prepared by diluting 6cc of concentrated HCl (1.19 specific gravity) with water to 500cc.

To the mixture of filtrate and acid add fibrin or egg albumin and set aside at 40° as above. If in both these tests the fibrin or albumin remains undissolved it is due to the absence of pepsin.

Each student will receive five "ukknowns" and these are to be tested for lactic acid, free HCl, and for pepsin. Report the results.

Pepsin is not present in the gastric gluid in atrophy of the mucous membrane of the stomach.

The flakes of coagulated egg albumin are best prepared by gradually pouring a dilute solution of the egg albumin, with constant stirring, into boiling water.

CHAPTER VI.

PANCREATIC SECRETION.

Cut up the fresh pancreatic gland into very fine pieces, or better pass it through an Enterprise fruit-press. The pulp thus obtained can be used direct, or mixed with several volumes of water.

Place about 10cc of the pulpy mixture in a small beaker, add 25cc of water and boil for about 10 minutes. Crush the hard, coagulated lumps in a mortar and return to the liquid. Reserve this for experiment 1.

1). Cleavage action on fats.--The fat or oil employed for this test should be strictly neutral. It can be obtained in this condition by the following process: Place about 10cc of the oil (cotton-seed oil or butter) in a small separatory funnel, add 20cc of water and render the mixture distinctly alkaline with NaOH. Then add an equal volume of ether and shake till the fat dissolves. Draw off the aqueous liquid and to the ether add an equal volume of waterand shake again to wash the ether. Remove the aqueous layer and wash once more with water. Transfer the ether solution, filtered, if need be, to a porcelain dish and allow the ether to evaporate. The neutral fat is left behind.

Place in each of two test-tubes about 3-4 cc of the neutral fat, 15cc of water and a few drops of concentrated aqueous blue litmus solution.

a). To one test-tube add about 5cc of the fresh pancreatic pulp mixture and shake.

b). To the second tube add one half of the liquid containing the boiled pulp and shake. If the contents of the two tubes react acid add, drop by drop, a Na_2CO_3 solution (2%) until the mixture is distinctly alkaline.

Place the tubes in an incubator at 40° for 6-8 hours, or over night. Compare the reaction of the tubes. Reserve the two mixtures for the next experiment. If the mixtures remain too long at this temperature bacteria develop and,giving rise to acids, reduce the litmus. The two tubes will then be quite alike and will give the same results in the next experiment.

Under the influence of a ferment in the pancreas, known as steapsin, or pialyn, the neutral fat is, in part, decomposed into free fatty acid and glycerin.

2). Emulsifying action on fats.--After digesting the two mixtures at 40° for 6-8 hours in the preceding experiment, shake thoroughly and take 1/3--1/2 of the contents of each tube andtreat as follows:

a). To a portion of the mixture from Exp. 1a. add about 1cc of Na_2CO_3 solution (2%) and shake thoroughly. The liquid becomes milky and on standing the fat does not rise to the surface. Examine a drop of the emulsion under the microscope.

b). To a portion of the mixture from Exp. 1b. add Na_2CO_3 solution as above and shake. The liquid does not emulsify. The fat rises rapidly to the surface on standing. If bacterial decomposition has taken place, or if the fat was not neutral in the beginning, some emulsification will result.

Why is the fat emulsified in one case and not in the other?

3). Diastatic action on carbohydrates.-- Prepare a starch paste by boiling 1g of powdered starch with 100cc of water. Place in each of two test-tubes 15cc of the starch paste.

a). To one add about 2cc of the pancreatic pulp mixture mix andplace in a water-bath at 40°.

b). To the second add 1/4 of the boiled pulp mixture and likewise set aside in the water-bath at 40°.

At intervals of about 15 minutes take out a portion, about 2cc, from each of the two tubes. Test one half of each portion with iodine for starch and dextrine. Test the remainder for sugar by boiling with Fehling's solution. How soon does dextrine make its appearance? How early can sugar be recognized? When is the achromic point reached?

Note the change in the appearance of the two tubes and the difference in results. Compare this action of pancreas with that of salive.

4). Proteolytic action.--In a 50cc Erlenmeyer flask, provided with a cork, place about 5g of fresh fibrin and about 20cc of chloroform water. This is prepared by adding 2cc of chloroform to 50cc of water and shaking thoroughly. To the fibrin and chloroform water add about 5cc of the pancreatic pulp and mix well. Render the mixture distinctively alkaline by the addition of a few drops of Na_2CO_3 solution (2%). Cork the flask and set aside at 40° for 2-3 days.

Occasionally examine the contents of the flask and compare th rate of digestion with that of gastric juice. Note that the fibr does not swell up as in gastric digestion, and that the edges are evenly eaten away.

The contents of the flask are finally slightly acidulated

with acetic acid, boiled and filtered. The filtrate may contain albumose, pepton, tyrosin and leucin.

a). To a portion of the filtrate apply the biuret test in the cold.

b). To another portion add a few drops of bromine water and shake. A pink to a purple-red color (proteinorchrom) develpps. This is known as the bromine reaction and is due to an unknown substance, proteinochromogen or tryptophan.

c). Concentrate the remainder of the filtrate on a wathh-glass to a small volume (a few cc) and set aside in a cool place over night. Examine the deposit with the microscope for tyrosin which forms characteristic bundels of needles, and for leucin balls. If no deposit forms concentrate to a thin syrup and again set aside for examination.

L E U C I N . $C_6H_{13}NO_2$.

This compound was formerly considered to be x-amidocaproic acid but the more recent studies of Schulze and Likieruik have shown it to be x-amido-isobutyl-acetic acid-- $(CH_3)_2CH.CH_2.CH(NH_2).CO_2H$. Leucine is readily formed and is a constant cleavage procuct, in the decomposition of proteids, gelatin and horn. This decomposition occurs in pancreatic digestion; may be brought about by the actions of acids and alkalies at high temperatures; and may also occur as a temporary bacterial product during putrefaction. Plant proteids, as well as animal proteids, can give rise to leucin It can be readily prepared from proteids, or better white horn, by boiling with dilute HCl. Leucin has been found, in diseased conditions, in various organs and glands of the body, in pus, blood, and in decomposed epidermis such as is found on the feet and between the toes. In the latter case the peculiar oder is largly due to decomposition products of leucin. This compound occurs with tyrosin, in the urine in liver diseases, especially in acute yellow atrophy.

Leucin is dextro-rotatory in acid or in alkaline solutions but in neutral solutions is inactive. On heating with baryta it becomes inactive. By the action of penicillium the latter variety is changed into a laevo-rotatory variety. Several isomeric leucins have been prepared synthetically.

In the pure condition leucin forms glistening white plates, which do not readily moisten when touched with water. As usually met with, however, it forms balls or aggregations of spherical bodies which often showslight radial marking and are faintly refractive to light. When impure leucin is more readily soluble than when pure. It is readily soluble in cold water (27 parts); more readily in hot water. It is difficultly soluble in alcohol, but is readily soluble in acids and alkalies. It forms salts with acids and bases.

The following experiments are made with leucin furnished by the laboratory:

1). To a drop of water on a slide add a little leucin, about the size of apin head. Observe the behavior on contact with water. Then mix, place on cover-glass and examine under the microscope. Sketch the crystals obsefved.

Then add a drop of water to the edge of the cover-glass and gently heat over a flame until the leucin dissolves. Set aside to cool slowly, then examine and sketch the crystals.

2.) To a few cc of urine in a watch-glass add a little leucin, mix and heat till it dissolves. Concentrate on the water-bath to a small volume; cover with a beaker and set aside over night. Then place the watch-glass under the microscope and examine with a low power. Typical light yellow spherules or mulberry-like masses of leucin will be found. Transfer some of the deposit to a slide and examine with a higher objective.

3). To about 1 c.c. of water in a test tube add leucin and shake till it ceases to dissolve. Divide into two portions:

a. To one add a drop of HCl, diluted.
b. To the other add a drop of dilute NH_4OH.

4). Plane some leucin in a glass tube, about 6 inches long, and open at both ends, and heat gently in an inclined position. A portion of the leucin is sublimed as a woolly deposit. At the same time an oder of amylamin is given off.

5). Dissolve some leucin in a little water and render the solution alkaline with Na OH. Then add 1-2 drops of dilute $CuSO_4$ solution. The cupric hydrate precipitate which forms at first, redissolves since with leucin it forms a soluble compound. The solution is colored blue and on heating does not reduce. This action of leucin is similar to that of glycocoll, of tarbrates, and of bile.

6.) Place in a dry test tube a piece of solid KoH about 1/2 inch long; add some leucin and a drop of water. Heat cautiously till the KOH melts. Place a strip of moist red litmus paper near the mouth of the tube. What is the result and to what is it due? Pour the melted KOH into a small beaker, rinse the tube with a little water, and add this to the beaker. Place the beaker in cold water and add very cautiously, drop by drop, H_2SO_4 till the solution is acid. Then heat the beaker over a flame and note the peculiar odor of valerianic acid. If the odor is not marked pour the liquid into a test tube, cork and set aside for a day or two. On opening the tube the odor will be perceptible.

7). Place some crystals of leucin on a platinum foil, add a drop or two of nitric acid (1.20 specific gravity) and evaporate carefully to dryness. A colorless scarcely visible residue remains. Now add a few drops of MaOH and warm. The residue dissolves forming a clear or slightly colored solution. On cautious concentration on oily drop remains which does not moisten the foil but rolls about readily. This is known as Schorer's test and is very characteristic.

For the detection of leucin in urine see Tyrosin.

TYROSIN, $C_9H_{11}NO_3$.

Tyrosin has been prepared synthetically and is therefore known to be para-oxyphenyl-alpha-amidopropionic acid,

$CH_2.CH(NH_2).CO_2H.$

Tyrosin is a constant cleavage product resulting from the action of tyypsin, bacteria, acids or alkalis on animal or vegetable proteids or horn. It is not obtained from gelatin or gelatin-yielding tissues. It is as a rule accompanied by leucin. It is present in old cheese and its name refers to this source. It is present in the intestines during proteid digestion but is not present in the tissues, bood or urine of the normal body. It is met with in the urine in phosphorous poisoning and in acute yellow atrophy of the liver.

It forms deli cate colorless silky needles which melt at 235°. The crystals often group in bundles and when very impure may form leucin-like balls. It is very difficultly soluble in cold water (1-2400), more soluble in hot water, insoluble in alcohol or ether. It is readily soluble in dilute alkalis and in dilute mineral acids. In acid solution tyrosin is laevo-rotatory whereas the synthetic re duct or that prepared by the aid of alkalis is dextro-rotatory.

The tyrosin necessary for the following experiments is furnished by the laboratory:

1). Treat a small portion in the same way as in experiment 1, under Leucin. How can the bundles of fine needles of tyrosin be distinguished from similar bundles of needles of fatty acids? of calcium sulphate?

2). Test the solubility of tyrosin according to the directions given in experiment 3 under Leucin.

3). To some water in a test-tube add a little tyrosin, then a few drops of Millon's reagent. Heat the liquid till it begins to boil. It colors rose-red, and on standing becomes dark-red and may yield a red precipitate. This is known as Hofmann's reaction and is due to the presence of the oxy-phenyl group in tyrosin. What other substances give this reaction with Millon's reagent?

4). Place some tyrosin in a dry test-tube, add a few drops of concentrated H_2SO_4. Place the test-tube in a water-bath and heat at 100° for about half an hour. Then cool and pour the contents into a small beaker containing some water. To this liquid now add $BaCO_3$ in small portions while stirring, until the reaction ceases to be acid. Filter the liquid and concentrate the filtrate to a very small volume. To this concentrated liquid add a drop or two of very dilute $FeCl_3$. A beautiful violet color develops. This test is known as Piria's reaction.

5). Place some crystals of tyrosin on a platinum foil, add nitric acid (1.2 sp. g.) and warm. The tyrosin becomes bright orange yellow and dissolves. Evaporate very cautiously to dryness when a deep yellow, transparent residue remains. Add a few drops of NaoH and a deep reddish-yellow solution results. This on evaporation leaves an intense blackish brown residue (Scherer's test).

A similar reaction is given by the other substances and consequently it is not characteristic.

6). To a boiling aqueous solution of tyrosin add some 1 per cent. acetic acid and then sodium mitrite solution, drop by drop. A beautiful red color develops (Wurster).

7). To a hot aqueous solution of tyrosin add some dry quinone. The liquid becomes colored a ruby-red (Wurster).

DETECTION OF LEUCIN AND TYROSIN IN URINE.

Tyrosin may occur in the sediment in urine but may be in solution. Inasmuch as leucin is more soluble it will be, as a rule, in solution in the urine.

Precipitate the urine with basic acetate of lead, filter and remove the lead from the filtrate by hydrogen sulphide. Then concentrate the solution as low as possible and set aside to crystallize. Examine under the microscope for crystals of leucin and tyrosin. If leucin is present it can be removed by means of warm alcohol.

CHAPTER VII.
THE BILE.

Bile is a mixture of the secretion of liver cells and of mucin derived from the cells lining the bile-bladder and duct. It is a thick, tenaceous fluid and is alkaline in reaction. The specific gravity ranges from 1.01 to 1.04. The color of bile varies in different animals. It may be light yellow, brownish yellow, brownish green, green, and greenish blue. Human bile is yellowish, at times greenish. Bile possesses a pronounced bitter taste. It does not coagulate on heating. Human bile contains ture mucin, whereas, ox-bile contains but traces of mucin and instead a muceoalbumin.

The quantity of bile secreted in 24 hours is subject to considerable variation even in health. In the case of fistulas, from 0.6 to 1 liter of bile has been observed to be secreted in 24 hours, but the secretion under these conditions can hardly be considered as normal bile. The actual quantity fiven off in a day is probably not less than a half a liter. After a proteid diet the secretion is increased, whereas, with fath and carbohydrates it is less marked The secretion is also decreased in starvation. The secretion is continuous but with variable intensity. Inasmuch as the bile flows from the bladder under very little pressure a slight obstruction in the duct may lead to retention of the bile. As a result the bile constituents are absorbed and may appear in the urine. Human bile as it is found in the bladder after death has been found to contain from 7-18 per cent. of solids. The bile as it flows from the liver, in a fistula, contains much less solids, 1-4 per cent. The bile, therefore, becomes concentrated in the bladder by absorption of water.

Bile contains as characteristic constituents certain salts of bile acids, bile pigments, and small quantities of lecithin, cholesterin, soap, neutral fat, urea, and salts of calcium, magnesium, iron, and copper.

Tje bile acids are usually present as sodium salts. In some sea-fish they are in combination with potassium. It is customary to speak of two bile acids, glycocholic and taurocholic. The former on cleavage yields glycocoll and cholic acid; the latter taurin and cholic acid. Inasmuch as this cholic acid is but one of several cholic acids known, it follows that there is a group of glycocholic acids, and a group of taurocholic acids. Human bile yields three cholic acids. The bile of some animals may contain only glycocholic acid, or only taurocholic acid; whereas, in some variable mixtures of the two acids are present. Thus, the taurocholic acid predominates in the bile of carnivorous animals, birds, reptiles, and fish. The bile from the rabbit and the hog contains almost entirely clycocholic acid. Herbivorous animals as a rule contain variable quantities of both acids. Both glycocoll and taurin are amido acids. Taurin contains S as a characteristic constituent. According to Hammersten the bile of some animals contain a third group of bile acids which are rich in S and which in their behavior to mineral acids resemble ethereal sulphates.

The bile-acid salts are precipitated from their solution in water or alcohol, on the addition of ether, as fine needles. The bile acids and their salts are dextro-rotatory.

A large number of bile pigments are known but in normal bile, as a rule, there are but two, bilirubin and biliverdin. The former can be obtained as a reddish yellow powder; the latter as a greenish powder. The color of the bile is due to the preponderance of one or the other of these two pigments. Ox-bile has both pigments. The other bile pigments as, bilifuscin, biliprasin and bilicyanin, have been isolated from bile stones and altered bile.

The bile pigments are soluble in alkalis, insoluble in acids, and yield insoluble compounds with calcium and other metals. Bilirubin is slightly soluble in alcohol and in ether, readily soluble in chloroform. Biliverdin is insoluble in chloroform. Bilirubin, in addition to being in the bile, is met with in bile stones as a calcium compound; in old blood extravasations (haematoidin) and in urine and tissues during icterus.

The source of bilirubin is undoubtedly haematin. On reduction it yields hydrobilirubin which is closely related, if not identical, with stercobilin (found in the intestines) and with urobilin of urine. On oxidation it yields biliverdin. The amount of pigment in the bile is usually only a few hundredths of a per cent., rarely 0.1 per cent.

As to the origin of these bile constituents it may be said that the bile acids are elaborated by the cells of the liver, not elsewhere in the body. The bile pigments, without doubt, can be formed in other parts of the body, than in the liver, but under normal conditions the liver is the organ where they are formed. Taurin and glycocoll result from the decomposition of proteids in any part of the body.

1). Place some dilute bile (1-5) in a test-tube and heat to boiling. Immerse a strip of red litmus paper, then remove and wash with water. The reaction is distinctly alkaline.

2). Place about 5 cc of bile in a test-tube, add 10 cc. of water; mix and filter if necessary. To the clear liquid add acetic acid. A cloudiness or distinct precipitate of mucin or nucleoalbumin forms on standing. This is not marked in ox-bile.

3). Filter the cloudy liquid obtained in Exp. 2, and apply the biuret test to the clear filtrate. Absence of proteids. Notice also, that the $Cu(OH)_2$ precipitate which forms redissolves in the bile solution and yields a blue liquid which on heating gives a black precipitate. What is the cause of this black precipitate? What other substances redissolve $Cu(OH)_2$ and yield blue solutions?

4). To about 20 cc. of bile in an evaporating dish add about 5 g. of animal charcoal and evaporate on the water-bath, with frequent stirring, to complete dryness. Transfer the residue to a 150 cc. Erlenmeyer flask, provided with a cork and condensing tube, add about 30 cc. of absolute alcohol and boil on the water-bath for about half an hour. Cool and filter into a dry flask (or a 50cc. test-tube on foot). To the alcoholic filtrate add anhydrous ether till a permanent precipitate forms. Then cork and set aside in a cool place over night. The sodium salts of glycocholic and taurocholic acids crystallize out. Filter off the crystalline deposit and save the filtrate. Squeeze the crystals as dry as possible on

the filter in the funnel, then dry between several sheets of filter paper. Save the crystals for subsequent tests.

5.) The alcohol ether filtrate from the preceeding experiment contains, among other things, cholesterin. Place this filtrate in an evaporating dish and allow the ether to evaporate spontaneously, then cautiously evaporate to dryness on the water-bath. Rub up the residue, thoroughly, with some ether, filter the ether solution into a small beaker or watch-glass, and allow the ether to evaporate spontaneously. Examine the residue under the microscope for the characteristic crystals of cholesterin. Fatty crystals, in the form of needles, are likely to be present.

6). DETECTION OF BILE ACIDS.

On the side table are two sets of diluted bile--bile-water, bile-urine--1-10, 1-100, 1-500, 1-1000. Apply the following tests first to the "bile-water" dilutions, then to corresponding dilutions of bile with urine. Tabulate the results.

Place about 5 cc. of each of these solutions in test-tubes and apply the following test, noting carefully the delicacy of the reaction.

a. To the liquid to be tested add about two-thirds its volume of concentrated sulphuric acid. The acid is allowed to run down the side of the tube slowly, so as not to mix. The temperature shouldnot rise over 60-70°. If necessary, therefore, cool partly under the hydrant, then add 2-3 drops of a solution of cane-sugar (1-10) and tap the tube gently. A pink to a red or violet color develops according to the amount of bile acids present. The foam which forms on shaking is likewise colored pink. This is known as Pettenkofer's test, and depends upon the formation of furfurol. An excess of sugar and too much heat must be carefully avoided Observe the difference in the delicacy of the reactions in aqueous and urine solutions of bile.

To some water in a test-tube add sulphuric acid as above, then about 5 drops of the sugar solution. Notice the yellow to a dark-brown color that forms. Repeat this blank test with urine, acid, and 5 drops of the sugar solution.

b. Furfurol Test.- Since Pettenkofer's test depends upon the formation of furfurol out of the sugar added the former can be added direct.

To a few cc. of the solution to be tested add one drop of a 1.0% aqueous furfurol solution, then add slowly as in the preceeding test about an equal volume of concentrated sulphuric acid, cool somewhat, if necessary, and avoid an excess of furfurol. The reaction is often less intense than in 6a.

Apply this test to some diluted bile. Dissolve a little of the crystallized bile acids obtained in experiment 4, in some water. Observe the foaming of the liquid when shaken. Divide this solution into two portions and test one according to Pettenkofer; the other with furfurol.

c). Detection of bile acids in the urine (Hoppe-Seyler's Method).-- The test given above under 6a is usually employed. It

should be remembered however that substances may be present in urene which will give a reaction similar to that of bile acids. Moreover, in highly colored urines the reaction can be readily masked. In such cases the following method of Hoppe-Seyler, though somewhat long, will give good results. About 100 cc. of the urine is evaporated to a syrup and the residue extracted with strong alcohol. The alcoholic filtrate is evaporated to dryness, and the residue obtained is dissolved in water. The aqueous solution is precipitaged with lead acetate and ammonia. The precipitate is wahed, then transferred to an evaporating dish or flask and extracted with boiling alcohol. The alcoholic solution is filtered while hot. A few drops of soda solution are added to the filtrate and this is then evaporated to dryness.

The dry residue can how be dissolved in water, the solution slightly acidulated with H_2SO_4 and filtered. The aqueous filtrate can now be tested directly for bile acids according to Ca or b.

7). DETECTION OF BILE PIGMENTS.

On the side-table will be found five bottles containing urine diluted with bile in the following proportions: 1-10, 1-20, 1-50, 1-100, 1-500. Apply the following tests to these solutions and tabulate the results.

a). Gmelin's Test.--Place some bile on the suspected urine in a small evaporating dish and add a drop or two of fuming HNO_3--a play of colors, green, blue to violet results. With ox-bile the colors are weak and rapidly change. In urine the green color is especially important since indican may also give a blue color. Various modifications of this test have been suggested and of these the following are especially useful.

a'. Filter the bile solution or suspected urine. Then add a drop or two of fuming nitric acid to the moist filter paper. The colored rings are very distinct. This test (Rosenbach's) is much more satisfactory than the preceding and is especially useful when the urine is highly colored.

a^2.) To a few cc. of fuming HNO_3 in a test-tube add slowly some dilute bile solution or the suspected urine so that the two liquids do not mix. Colors develop at the zone of contact. Finally mix the contents; a decided green color forms, especially on standing.

b. Huppert's Reaction.-- To about 10 cc. of the diluted bile or suspected urine add a little calcium chloride, then an excess of ammonium or sodium carbonate. The bilirubin--calcium compound is precipitated. Filter, wash the precipitate then transfer while moist to a test-tube and fill it half full of alcohol which has been acidulated with sulphuric acid. Immerse the tube for 10-15 minutes in a water-bath, heated, so that the contents of the tube are kept near the boiling point. The solution becomes colored an emerald to a bluish green. Now cool the contents of the tube, then add fuming HNO_3. The green color changes to blue, violet, and red. This test is very delicate and is especially useful when the urine is highly colored, or contains much indican or blood pigments.

c. Iodine test.-- Place the diluted bile or suspected urino om a test-tube, incline the tube and add cautiously 2-3 cc. of a dilute tincture of iodine so that it forms a layer. Immediately or after a few minutes a bright green ring forms at the zone of contact (Rosin-Smith). This reaction is almost as delicate as that of Huppert.

d.) Jolle's Test.- This is claimed to be the most delicate test for bile pigments. Place 50 cc. of the suspected urine or a mixture of bile and ur ing (1-500) in a glass stoppered cylinder, add a few drops of 10% HCl, then $BaCl_2$ in excess and 5 cc. of chloroform and shake vigorously for several minutes. Set aside for about ten minutes for the precipitate and chloroform to settle. Transfer the chloroform and precipitate by means of a pipette to a test-tube. Immerse the tube in a water-bath having a temperature of about 80°. The chloroform evaporates in about 10 minutes. Remove the test-tube and after a few minutes when the precipitate has bettled decant the supernatent liquid. The precipitate is colored yellow if bile pigment is present. Allow 3 drops of concentrated HNO_3 (to which about 1/3 fuming HNO_3 has been added) to run down the side of the tube. The characteristic play of colors develops.

e.) Acidulate some dilute bile with acetic acid, add a few cc. of chloroform and shake. The chloroform dissolves the bilirubin and is colored yellow.

BILE STONES.

The calculi found most often in the gall-bladder of man consist chiefly of cholesterin. They may be grayish or yellowish white, wax-like in appearance, or may be colored from a light red to a dark brown. The color depends upon the amount of bilirubin present. This pigment is not free but in combination with calcium. The number of stonespresent in the gall-bladder may vary from a few to several hundred. The size will, therefore, vary considerably, from that of a grain of wheat to stones from 1/2-1 inch in diameter. As a result of friction the stones frequently show smooth triangular faces. The larger stones when cut in two and polished show generaly a concentric arrangement. When they consist of pure cholesterin the stones will float on water. Small amounts of fat may also be present.

The bile-stones as usually found in the gall-bladder of cattle consist largely or wholly of the calcium-bilirubin compound. Similar calculi are met with occasionally in man. These pigment stones may contain metals such as iron and copper and even at times zinc and manganese. Unlike the cholesterin stones they are always heavier than water.

A third form of bile-stone very rarely found in man consists chiefly of calcium carbonate and phosphate.

EXAMINATION OF BILE STONES.

Pulverize a small bile-stone and pla ce the powder in a test-tube. Add a mixture of alcohol and ether, equal parts, and warm gently until the powder ceases to dissolve... Decant the ether-

alcoholic solution onto a watch-glass or evaporating dish and allow it to evaporate spontaneously. If the crystals are imperfect redisolve in hot alcohol and allow the solution again to evaporate spontaneously.

Save the crystals for the subsequent tests for cholesterin.

If there is a residue insoluble in the ether-alcoholic mixture add to it some dilute HCl. An effervesence indicates a carbonate ($CaCO_3$). If an insoluble residue still remains wash it with water and examine for bile pigments.

Evaporate the HCl solution to dryness and ignite; then dissolve the residue in dilute HCl and add NH_4OH. A blue color indicates the presence of copper.

Cholesterin, $C_{27}H_{45}OH$.--is a common constitutnt though in minute quantity of the normal fluids and tissues of the body. Under pathological conditions it is met with especially in bile stones. It is also present in atheroma nodules, in tubercular masses, tumors, sputum, pus transudates and cystic fluids. It is rarely present in the urine and then in small amount. A rare urinary cholesterin calculus has been reported by Horbuesewski. Compounds closely resembling cholesterin, possibly isomers, are found in plants (phytosterius).

Cholesterin forms white, glistening crystals which under the miscroscope appear as evry thin transparent plates with a corner more or less notched. The crystals melt at 145° whereas plant cholesterin melts at 133°. It is insoluble in water, in dilute acids, and in alkalis. It is readily soluble in boiling alcohol from which on cooling it recrystalizes. It is readily soluble in ether and chloroform.

1). Examine under the microscope and sketch the characteristic crystals of cholesterin obtained from a bile stone.

2). To some crystals on a slide under the miscroscope add a drop of dilute H_2SO_4 (5 parts of acid to one part of water) The edges of the crystals show a bright carmine red color which changes to violet.

3). To some crystals as in Exp. 2, add a drop of dilute H_2SO_4 then a drop of iodin solution. The crystals turn gradually violet, bluish green, then blue.

4). Dissolve a few crystals in a little chloroform in a dry test-tube, then add an equal volume of sulphuric acid and shake. The chloroform becomes blood red, then cherry red and purple. The acid liquid shows a green flouresennce (Salkowski). The color of the chloroform is quickly discharged if it is poured into a moist test-tube.

5). Dissolve some cholesterin in 2cc of chloroform, add 10 drops

of acetic anhydride and then drop by drop, concentrated H_2SO_4. The mixture becomes red, blue and finally green (Liebermann's Cholesterol reactionT.

6. To a little cholesterin in an evaporating dish add a few drops of HCl, and a drop of very dilute $FeCl_3$. On evaporating to dryness a blue color results.

7). Place a little of the dry cholesterin in a dry test-tube and add 2-3 drops of propionic anhydride and carefully heat over a small flame till melted. On gradually cooling the mass becomes violet then green, blue and red.

Detection of cholesterin in urine.--

Inasmuch as cholesterin is lighter than water it will be found whan present in urine, floating on the surface as a thin pellide. Some crystals may be dragged mechanically to the bottom. A microscopic examination will ofter decide the nature of this film. This is also true of transudates and other pathological fluids where the crystals are often well formed. In the absence of typical crystals it will be necessary to employ the following method.

Extract the urine with ether which takes up fat and cholesterin. Remove the ethereal layer and allow it to evaporate spontaneously. Examine the residue under the microscope for the characteristic crystals of cholesterin. If there is any doubt owing thethe presence of fats these must be removed by saponification. For this purpose dissolve the residue in hot alcohol, add some strong alcoholic solution of sodium hydrate and heat on the water-bath for some time. Finally evaporate to dryness, and extract the residue of soaps with ether. This ethereal solution on evaporation will now give a residue free from fat.

CHAPTER VIII.

B L O O D

I. MICROSCOPIC EXAMINATION.

1). Examine a drop of fresh blood under the microscope. Measure the diameter and sketch the red and white blood cells. What is the difference between the blood cells of mammals and of birds, reptiles, etc.

2). Dilute some fresh blood with water and examine as before. Observe and sketch the crenated blood cells.

II. SPECTROSCOPIC EXAMINATION.

1). Add 1cc of defibrinated blood to 50cc of destilled water and shake thoroughly. Place some of the dilute blood in a test-tube and suspend this about an inch above the slit of the spectroscope (Position No. 1.) The test-tube should not be more than one half inch in diameter. A fish-tail burner placed about 3 inches from the slit serves as a source of light.

Observe the two absorption bands of oxy-haemoglobin and their postion on the scale in the spectrum.

Place in the flame a platinum wire previously dipped in a solution of sodium chloride. Notice the characteristic yellow line of sodium, its postion on the scale and its relation to the two absorption bands.

2 . Now swing into position the little outside prism of glass so that it shuts off the lower half of the slit. Place a light about 3 inches in front of the left face of this prism. The spectrum of haemoglobin appears in the lower half while superposed above it is a clear spectrum. Place a tube of the blood, diluted and well shaken as above, between this second light and the left face of the prism (Position No. 2.). The spectrum from this tube is now thrown above that from the tube in front of the slit, The two spectra of ohy-haemoglobin coincide.

a). To tube No. 1 before the slit, add 1-2 drops of freshly prepared ammonical ferro-tartrate solution (Stokes' solution), and examine at once. The two bands of ohy-haemoglobin soon disappear giving place to the single wide band of reduced haemoglobin Compare this spectra with the superposed one of ohy-haemoglobin. Note the change in the color of the tube.

~~The Stokes' solution is prepared as follows: Dissolve 2 parts~~

of ferrous sulphate and 3 parts of tartaric acid in water. then render alkaline by addition of NH_4OH.

b). To tube No. 2. the one on the left, now add 5-6 drops of strong ammonium sulphide and examine. Ina few minutes the single band of reduced haemoglobin takes the place of the two bands of oxy-haemoglobin. The spectra of the two tubes now conincide.

3). To the tube of reduced haemoglobin in position No. 1 obtained in experiment 2a, add a few drops of concentrated NaOH. The single absorption band becomes replaced by two bands, resembling those of oxy-haemoglobin but shifted a little to the right. The left band is the darker of the two. On standing a few minutes the spectra increases in intensity so that the two bands merge together; in that case dilute with an equal volume of watdr. and examine again.

This spectrum is due to haemochromogen or reduced haemoglobin This test should be resorted to when the spectrum of haemoglobin is doubtful.

Compare this spectrum with the superposed spectrum of reduced haemoglobin (2b) . Then substitute for the latter a tube of the diluted, well shaken blood, thus placing the spectrum of oxy-haemoglobin above that of haemochromogen.

4). Dilute some defibrinated blood with about 15 parts of water and shake well. Place some of this solution in a test-tube, in postion 1. Superpose the spectrum of oxy-haemoglobin using dilute blood as in experiment 2 (1-50). The upper spectrum of the very dilute blood shows the two bands of oxy-haemoglobin. whereas, the lower spectrum, tube No. 1 which contains a strong solution, is entirely dark to the right of the soldium line.

To the tube in front of the slit, position 1, now add 1-2 drops of a fresh, concentrated solution ofpotassium ferrocyanide. The color of the liquid changes to a brown and the spectrum of Methaemoglobin appears. An intense dark band in the red with two less dark bands in the rignt. If the liquid is too concentrated dilute 1/4--1/3 with water.

To the so,ution of Methaemoglobin add a few drops of $(NH_4)_2S_2$. The color and spectrum of oxy-haemoglobin reappears, and in a short time this gives way to that of reduced haemoglobin(2b).

5). To about 10cc of concentrated H_2SO_4 in a test-tube add about 5 drops of blood. Shake thoroughly after the addition of each drop of blood and keep the contents of the tube cool. Note the dark wine red color of the solution.

Dilute a portion of this liquid with 2-3 parts of water,

cool and examine before the spectroscope (position 1) for the spectrum of haemotoporphyrin. Superpose as in experiment 2 the spectrum of oxy-haemoglobin (1-50) for comparison. Haemotoprophysin shows a dark narrow band to the left and a wider, darker band to the right of the left band of oxy-haemoglobin.

Haemotophophysin, $C_{16}H_{18}N_2O_3$ is derived from haematin by the splitting off of iron. It results also from the action of HBr on haematin. It is an isomer of bilirubin and has been met with in urine.

6). To 5cc of diluted blood (1-15) add 2cc of concentrated NaOH. The color changes to a cherry rer. Now heat the tube till the color changes to a brownish green. Examine before the spectroscope, position 1, for the spectrum of alkaline haematin. If necessary dilute the contents of the tube 1/4--1/3 with water. Alkaline haematin shows a dark band through the middle of which passes the sodium line Superpose the spectrum of oxy-haematin and conpare the two spectra. Then convert the upper spectrum into reduced haemaglobhin as in experiment 2a and again compare.

7). Pass a current of illuminating gas some diluted blood (1-50).

a). Place a tube containing some of the blood thus treated before the spectrum in position 1. Superpose the spectrum of oxy-haemoglobin (1-50). The lower spectrum, due to carbon monoxide haemoglobin, is nearly the same as that of oxy-haemoglobin. The two bands, however, are darker and are removen a trifle to the right so that the two spectra are not exactly continuous. Compare the color of the two tubes.

b). Now add to each tube 1-2 drops of Stokes' solution. Carefully note the change in color of the two tubes and also the change in the spectra.

c). Again superpose the spectrum of oxy-haemoglobin above that of CO-haemoglobin Then add to each tube 5-6 drops of strong $(NH_4)_2S_2$. Examine at once and after the lapse of about 5 minutes.

d). Again superpose the spectrum of oxy-haemoglobin above that of CO-haemoglobin. Then add to each tube one drop of a frechly prepared strong solution of potassium ferricyanide. Examine at once. The oxy-haemoglomin spectrum changes in a few seconds to that of methaemoglobin whereas the spectrum of CO-haemoglobin persists and is changed only after the lapse of several minutes. Owing to the dilution the spectrum of methaemoglobin will be faint.

CO-haemoglobin is a much more stable compound than oxy-haemoglobin and for that reason the color and the spectrum of the solution in experiments b, c, and d, will change slowly, if at all, whereas oxy-haemoglobin is readily changed to reduced haemoglobin in experiments b and c, and to methaemoglobin in experiment d.

DEFIBRINATED BLOOD.

III. GENERAL REACTIONS.

1). Test the reactions of some fresh defibrinated blood.

a). Dip a moist red litmus paper for a few seconds into the blood, then wash at once in water.

b). Place a drop of aqueous red litmus solution on a porous porcelain plate. When this has been absorbed apply a drop of blood to the spot and allow this to remain for about a minute. Then wash off with water. Owing to the coloring matter in the blood this method of testing is much more delicate than the prededing.

2). To some water in a test-tube add a drop or two of blood and mix. Then add tincture of guajac till the liquid becomes cloudy, and finally add some old oil of terpentine. A blue color develops at the zone of contact of the liquids and is due to the oxidation of the guajac. The reaction fails with fresh oil of terpentine owing to the absence of ozone.

a). This test may be applied to urine, suspected of containing blood, in the following manner: Place in a test-tube equal volumes of guajac and old oil of terpentine. The mixture must not be blue. Now add the urine cautiously so that it forms a layer. If blood is present a bluish-green ring will form at the zone of contact. This is known as Almen's Guajac Test. The urine if alkaline should be neutralized or rendered faintly acid. Pus may give the test with guajac alone.

3). To 2cc of fresh blood in a test-tube add, without shaking, 2-5cc of hydrogenperoxide. Oxygen is liberated abundantly and the liquid foams and the haemoglobin is gradually decomposed. This is due to a so called catalytic action.

4). To some fresh diluted blood (1-5) is a test-tube add ether and gently agitate. The liquid becomes transparent because of the solution of blood cells--laky blood.

5). Pass a current of illuminating gas for a few minutes through some dilute blood (1-50). Notice the cherry-red color of the solution. As shown above in Exp. II 7, CO-haemoglobin is a much more stable compound than oxy-haemoglobin. The following tests still further serve to demonstrate this fact and are of great value in distinguishing between the two forms of haemoglobin. The tests c and d are especially adapted for the detection of small amounts of CO-haemoglobin in blood.

a). In one test-tube place some dilute blood (1-50); in another

some of the CO-haemoglobin solution (1-50). To each of these solution add half a volume of strong NaOH solution (1.34 specific gravity). The pure blood solution becomes brownish (due to haematin) whereas the CO-haemoglobin solution is unaltered and retains its cherry-red or pink-red color (Hoppe-Seyler).

b). Place 5cc of the diluted blood (1-50) in a test-tube. In another tube place 5cc of the CO-haemoglobin solution (1-50). To each tube add an equal volume of fresh, saturated H_2S-water and shake. The pure blood changes to a green, due to the formation of sulphur-methaemoglobin, whereas the color of the methaemoglobin is unchanged of fades slowly.

c) . In one test-tube place 5cc of the dilute blood (1-50); in another tube 5cc of the CO-haematin solution. To each of the tubes add 1-2 drops of dilute acetic acid, then one drop of potassium ferrocyanide solution (1-5). The proteids in both solutions are precipitated but the precipitate in the tube of pure blood is brownins, in color, whereas that in the CO-haemoglobin tube is pink. On standing a while the pink color changes and both precipitates are then alike.

d). In one test-tube place 5cc of the dilute blood (1-50); in another 5cc of CO-haemoglobin solution (1-50). To each of these tubes add an equal volume of freshly prepared 1% solution of tannic acid. The proteids are again precipitated. The precipitate in the tube containing pure blood is colored or grayish brown whereas that in the CO-haemoglobin tube is pink. An excessof tannic acid may dissolve the precipitate and should therefore be avoided.

Make a mixture of 1cc of CO-haemoglobin solution (1-50) and 4cc of oxy-haemoglobin solution (1-50) add an equal volume of the tannic acid solution and compare with the two tubes obtained above.

Haemoglobin is readily decomposed on heating with acids or alkalis into globulin and a pigment. If oxy-haemoglobin is acted upon the pigment that results is haematin, whereas with reduced haemoglobin the product is haemochromogen. The latter decomposition has been studied in experiment II 3, whereas the formation of haematin has been observed in II 6 and III 5a. Haematin combines with HCl to form haemin.

6). Preparation of haemin crystals.--Place in a small Erlenmeyer flask (about 30cc capacity) provided with a cock and condensing tube, 10cc of glacial acetic acid and heat to boiling on the water-bath for about half an hour. Then add, gradually and with constant stirring, 3cc of defibrinated blood. Continue heating on the water bath for half an hour. Transfer to a small narrow beaker or test-tube and set aside night. Examing the crystalline deposit microscopically and sketch the form og the haemin crystals.

To preserve the specimen, decant the acetic acid; then add 10-20 cc. of water, stir thoroughly and place aside to settle. Decant off the water and wash in a similar manner with alcohol; then stir up the crystals with ether and transfer to a small filter. Press the crystals between filter paper till dry, then transfer to a specimen tube.

The operation of washing can be greatly simplified by the use of a centrifugal apparatus.

The recognition of haemin crystals is of the greatest importance in the identification of blood stains. Each student will receive a piece of fabric and a piece of wood stained with blood. These are examined in the following manner:

a). Scrape a little of the stain off the piece of wood. Place the scrapings on a glass slide, add a drop of 1% solution of NaCl and warm gently over a very small flame, avoiding ebullition, until the water is nearly driven off. Then, while still moist add 1-2 drops of glacial acetic acid, cover with a cover-glass and again warm gently over a small flame till most of the acetic acid has evaporated. When cool, examine under the microscope for the characteristic light brown haemin prisms. Sketch the form of the perfect crystals.

b). Soak the cloth in a 1 per cent. solution of NaCl in a watch glass and squeeze out the coloring matter as thoroughly as possible. Concentrate the liquid, if it is but weakly colored, on the water-bath to a small volume. Then place 1-2 drops of the liquid on a glass slide, warm gently, as above under a, until the liquid is nearly evaporated then add 1-2 drops of glacial acetic acid, cover with glass and again heat gently till most of the acetic acid has evaporated. Cool and examine for haemin crystals.

7). The formation of haematin and of haemin crystals may be utilized for the detection of a small amount of blood or blood pigment in the urine. To the suspected urine add NaOH and boil. The earthy phosphates are precipitated and are colored brownish red by the haematin (Heller's test). If there is doubt as to the nature of the coloring matter in the precipitate, this can be filtered off and subjected to the haemin test according to the directions given above under 6a.

a). The urine may be precipitated with tannic acid and the precipitate can then be treated for haemin crystals as above.

8). Place about 20 cc. of defibrinated blood in a beaker and add about 200 cc. of water. Acidulate very slightly with acetic acid, boil and filter. The filtrate should be water-clear. Notice the brown color of the coagulum. To what is it due? Evaporate the filtrate to a small volume, about 20 cc. If a precipitate forms during the evaporation it should be filtered off. Test the clear concentrated liquid as follows:

a). Boil some Fehling's solution in a test-tube, then add some of the liquid and boil again. A yellowish red precipitate of cuprous oxide indicates the presence of sugar.

b). Acidulate a little of the liquid with HNO_3 and add some $AgNO_3$. A heavy white precipitate soluble in NH_4OH indicates the

presence of NaCl.

c). Acidulate another portion with HNO_3 and add some ammonium molybdate solutions. On gently warming a yellowish precipitate or coloration indicates phosphates. The test for phosphoric acid can be made by adding NH_4OH to the liquid, then magnesia mixture. A white cloud or precipitate forms if phosphoric acid is present.

d). Evaporate the remainder of the liquid in a watchglass on a water-bath till only a few drops remain. Then set aside to cool and examine under the microscope for crystals of NaCl.

BLOOD SERUM.

IV. PREPARATION OF BLOOD SERUM.

The blood is received, directly from an animal, into a wide cylindrical vessel or into a common fruit-jar. It clots in a short time forming a solid coagulum. The vessel is then placed in an ice-chest for 36-48 hours. As the clot shrinks the clear yellow serum is squeezed out and collects on the top. This yellow serum is removed with a pipette and is used for the following experiments. It not infrequently happens that the serum as obtained is reddish due to the presence of blood corpuscles. In that case it is best to place the serum in a tall, narrow beaker and set it aside in the ice-chest for 1-2 days when the corpuscles will subside and leave a straw-yellow, clear serum above.

Blood plasma, the liquid portion of the living blood, contains at least three proteids--fibrinogen, serum albumin, and serum globulin. In the process of clotting the fibrinogen is changed to fibrin and hence the blood serum contains the two proteids serum albumin and serum globulin, or paraglobulin.

Carefully review in this connection the work done on the proteids of blood serum ( ). What is precipitated if blood serum is saturated with $MgSO_4$? With $(NH_4)_2SO_4$?

1). Determine the coagulating point of undiluted blood serum (5-10 cc.) according to the method given under egg-albumin, Exp.21. Note the temperature at which the contents of the tube become cloudy; when they gelatinize and when they become solid.

2). In each of three tubes place one cc. of blood serum. To tube 1 add nothing. To tubes 2 and 3 add 5 and 10 cc. respectively of distilled water. Immerse in a boiling water-bath for 10 minutes Note the result. No. 1 coagulates solid whereas Nos. 2 and 3 do not.

Sufficient dilution of serum with water renders it non-coagulable by heat. If tepid-water is used, owing to the presence of calcium salts, partial coagulation will take place.

3). To each of 4 tubes add 1 cc. of blood serum; then add to each 10 cc. of distilled water. To tubes 1 and 2 add 1 and 5 drops respectively of a 1% acetic acid; to tube 3 add a couple of drops of $CaCl_2$solution; to tube 4 add 2 g. of NaCl. Immerse the tubes in a boiling water-bath for 10 minutes. Note the results and explain the same.

As shown above in experiment 4 blood serum diluted with 10 parts of water does not coagulate on heating. In experiment 5 tube 1 does coagulate whereas tube 2 does not. To tube 2 now

add 1cc of a 10% NaCl solution and boil it coagulates at once.

Tube 5 contains a fibrinous coagulum whereas tube 4 coagulates solid.

What effect would the addition of NaCl to serum have on the coagulating point?

Compare carefully this and the preceding experiment with experiment 7 and 8 on albumen. As shown beforeeven slight excess of acetic acid tends to prevent precipitation of albumin and globulin, whereas NaCl favors precipitation.

4). To 5cc of blood serum in a test-tube add 1 drop of formalin, mix and boil. The blood serum does not coagulate.

5). To 45cc of water in a small beaker add 5cc of blood serum; mix and filter. Receive the filtrate in a 50cc graduate and place this in a beaker of cold water. Pass a current of CO_2 through the diluted serum for about 15 minutes. Then cork and set aside in cold water for some hours. The paraglobulin is thrown out of solution as a fine cloud and eventually settles to the bottom as a white precipitate.

6). To 50cc of water add 1cc of blood serum and mix. To this dilute blood serum apply the following tests:

a). To about 10cc of the diluted serum add 1-2 drops of strong HNO_3. The cloudiness that forms disappears on shaking. Now heat the contents of the tube to boiling. A yellowish color develops but no coagulation takes place. Divide the liquid into two portions.

(1) Cool one portion then add 5-6 drops of NHO_3 and boil. Coagulation results.

(2) Raise the other portion to boiling then add 5-6 drops of NHO_3 and boil. Coagulation likewise results.

b). To 5cc of the diluted serum (1-50) add an equal volume of water. This gives a serum diluted 1-100. To this very diluted serum add a drop of strong HNO_3 and boil. No coagulation. Divide the liquid into two portions.

(1). Cool one portion then add 5-6 drops of HNO_3 and boil.

(2). Raise the other portion to boiling then add 5-6 drops of HNO_3 and boil. The solution remains clear.

Compare these two experiments and explain the difference in results.

7). To 5cc of the diluted serum (1-50) add 5-6 drops of strong HNO_3. A cloudiness forms and on boiling coagulation takes place.

a). Repeat this experiment with serum diluted as above under c, (1-100). Coagulation takes place as in the case of the 1-50 serum.

8). Boil 5cc of the dilute serum (1-50) and while boiling hot add 5-6 drops of HNO_3. Coagulation results. Compare the volume of the precipitate with that obtained in experiment 6a and 7.

a). Repeat this experiment with a serum diluted as above under b (1-100) only a slight precipitate forms. Compare the volume of the precipitate with that obtained in experiment 6a and 7a. Explain.

It is evident from the above experiments that in the heat and HNO_3 test for albumen, in the urine or elsewhere, it is necessary to take into account the amount of HNO_3 added and whether the solution is cold or h ot. The best result is obtained therefore when albumin is present in minute quantities, by adding to the cold solution an excess of HNO_3 (5-6 drops) to a permanent cloudiness and then boiling when a coagulation results.

HNO_3 and heat will coagulate albumin where heat alone will fail to do so. This may be the case if the urine tested has an alkaline reaction. An additional advantage in the use of HNO_3 is that it will dissolve any phosphates that may be thrown out of solution by heating the urine.

9). To 5cc of the diluted serum (1-50) add 1 drop of 1% acetic acid (1cc of glacial acid diluted to 100cc of water). A cloudiness results. Test the reaction of the liquid then boil. A coagulum forms and the liquid is perfactly clear.

a). Repeat this experiment, first raising the dilute serum to boiling and adding 1 drop of the 1% acetic acid. What is the result?

10). To 5cc of the diluted serum (1-50) add 3-4 drops of dilute acetic acid used above. Test the reaction of the liquid, then boil. No coagulation takes place but the liquid is opalescent.

a). Boil 5cc of the diluted serum (1-50), and while boiling hot add about 10 drops of 1% acetic acid and boil again. The cloudiness that forms at first, redissolves.

In precipitating proteids, from urine or other solutions, by means of acetic acid and heat care must therefore be taken to add the acetic acid to the cold solution to neutralization and after that to heat to boiling. ~~Even a slight~~ acidity due to acetic acid will ~~keep albumin~~ in solution.

Compare the behavior of acetic and hitric acids to the serum proteids.

11). To some of the dilute serum (1-50) add 1-2 drops of $HgCl_2$ A white precipitate forms. Shake up thoroughly and divide into two portions.

a). To one portion add an equal volume of NaCl solution (1-10) The precipitate promptly dissolves.

b). To the other portion add an equal volume of undiluted serum. The precipitate likewise promptly dissolves.

The precipitate of mercury and albumin is therefore soluble in NaCl, also in excess of proteids. Of what importance is this fact in practical disinfection? Compare this test with the similar experiment on egg albumin I, 4. Note the difference in the behavior of the two proteid solutions.

12). To some of the dilute serum (1-50) add dilute $CuSO_4$ solution till a precipitate forms. Then add a few drops of strong NaOh solution (1-5). The precipitate redissolves yielding a blue solution.

What other substances give similar solutions of cupric hydrate?

If silver nitrate or lead acetate be added to the dilute serum what would be the result? What is the behavior of the salts of heavy meatls to proteids?

The reaction given by serum ablumin and serum globulin as wotked out in the table, will of course be given by the diluted blood serum.

V. FIBRIN.

The coagulum obtained by whipping freshly drawn blood is cut up into small pieces and washed in running water till perfectly white

Fibrin on contact with dilute HCl at 40° swells up and the contents of the tube become solid in a few minutes, (See exp. 1, peptic digestion). Solution then gradually takes place so that in 2-3 days the fibrin has disappeared. An acid albumin results, (See Exp . 4).

Fibrin swells up also in 5% oxalic acid solution but does not dissolve readily. It is also soluble in dilute neutral salt solutions.

Place in each of two test-tubes about 5cc of hydrogen peroxide. To one add a shred of fresh fibrin. Oxygen is set free especially on slight warming through so called catalytic action. This action is probable due to remnants of leucocytes (nucleotuston)

To the other tube add some boiled fibrin. What is the result?

CHAPTER IX.

M I L K.

Milk is a secretion of the mammary gland. It is composed of water, casein, globulin, albumin, fats, milk-sugar, and inorganic salts . The color of milk is due in part to the suspended fat globules, and in part to the casein which is held in solution by calcium phosphate. The specific gravity of milk from a single animal may vary considerably, usually from 1,028 to 1,035, but may be as high as 1,039. Market milk which is the mixture of the product of several animals always ranges from 1.029 to 1.034.

The reaction of milk is usually alkaline or amphoteric. It may however, be said and this is especially true of carnivorous animals. On standing milk becomes gradually acid owing to the formation of lactic acid by fermentation. Fresh milk does not coagulate on heating. After fermentation sets in milk may coagulate on heating. and later curdles without the application of heat. Sterlized milk, prpperly kept, will remain sweet indefinitely. The scum which forms on boiled milk is not coagulated albumin but a combination of casein and calcium. When removed a new scum forms on the milk when heated. Solutions of casein under similar conditions become covered with scum.

The addition of rennet to milk produces in a short time a solid coagulum, the curd or cheese. The clear liquid remaining is the whey or milk-serum. The reaction of the milk is not affected by this change. The presence of calcium is necessary to the formation of curd. The casein originally present in the milk is apparently changed by the ferment into two proteids. One of these unites with calcium to form the curd and is known as paracasein. The other proteis is formed in small amount, is related to the albumoses, and is known as whey-proteid.

Casein is a complex proteid belonging to the nucleoalbumins. It is insoluble in water but readily dissolved in the presence of alkalis. A solution in calsium hydrate can he neutralized with phosphoric acid without precipitation of the casein. The milky liquid thus obtained contains, in solution or suspension, the casein and considerable calcium phosphate. Casein is thrown out of solution by dilute acids, or by saturation with NaCl or $MgSO_4$. It is also precipitated by metallic salts. In the presence of calcium a solution of casein is coagulated by rennet. As in the case of milk, a solution of casein when boiled becomes covered with a scum. On digestion with pepsin it yields pseudonuclein which contains phosphorous. The casein in woman's milk is different from that in cow's milk. The former is more diffficult to precipitate with acids, salts and rennet. When precipitated by an acid the coagulum is finely flocculent and dissolves readily in an excess of acid whereas casein from cow's milk is coarsely flocculent and is less readily soluble in excess of acid. Unlike casein from cow's milk it does not yield pseudonuclein on

digestion. Casein is derived apparently from a nucleoprotein contained in the protoplasm of the cells of the gland.

The globulin of milk, or lactoglobulin of Sebelian, is probably identical with serum globulin. Lactoglobulin is related to but not identilal with serum albumin. Like casein and milk-sugar it is a special product of the cells of the gland. Schlossmann found the three proteids present in milk in the following quantities: casein, 3.19%; albumin, 0.36%; globulin, 0.15%. Only traces of urea, creatin, etc. are normally present in milk, consequently all the nitrogen present in milk can be considered as contained in the proteid substances.

The fat is present as an emulsion, in the fat globules. These vary in size in milk from the same species and from different species. According to Woll they are on an average 3.7 μ in diameter and from 1-to 5.7 million's of globules are contained in one cc of milk. The former belief that the fatty globules were surrounded by an albuminous envelope is no longer held. The fat is supposed to result from a degeneration of the protoplasm of the cells but it is possible that a part, at least, is brought to the gland by the blood.

The sugar present in the milk, lactose, is a specific product of the gland cells and is not directly derived from the blood. It is possible that it is derived, like casein, from the nucleoproteids in the cells. That these compounds can give rise to carbohydrates has been demonstrated. In exceptional cases milk-sugar may appear in the urine. Like glucose it is dextro-rotatory and reduces Fehling's solution. Although readily decomposed by bacteria it is not acted upon by pure yeast. This fact as well as its solubility, crystalline form and the formation of mucic acid on oxidation with nitric acid distinguishes lactose from glucose.

The colostrum corpuscles can be considered as epithelial cells which have taken up fatty globules, rather than as degenerated cells. They are found in milk secreted just before and after delivery. And appear as nucleated, granular cells containing numerous fatty granules. They are from 5 to 25 μ in diameter. The milk at this time is yellowish in color, alkaline in reaction, and has a high specific gravity 1.046--1.080. When such milk is heated it coagulates owing th the presence of increased quantities of albumin and globulin. See analysis.

1). Examine a drop of milk under the microscope. Sketch the different sized globules present and measure their diameter. They average about 5 μ but some globules may attain a diameter of 18 μ or more.

2). Examine microscopically a drop of skimmed milk. What difference is observed between this and whole milk.

3).Examine with a microscope colostrum milk. Sketch and measure the colostrum corpuscles.

4). Place about 10cc of milk in a test-tube and boil. Then immerse litmus paper in the hot milk for 1-2 minutes, remove and examine. Under what conditions does milk become acid?

5). Boil about 25cc of milk in a small beaker for 5 minutes. No coagulation proper but a scum may form. Remove the scum with a spoon or spatula and heat again, a new scum forms. This removal of scum will repeatedly take place. What is the nature of the scum? Casein is not coagulated by heat. Why does not the albumin in the milk coagulate? Save the milk for experiment 13.

6). To about 10cc of milk in a test-tube add 1 drop of dilute acetic acid (L-10), then boil. The casein is coagulated and carries down with it the fat. The serum is clear.

7). Set aside in a test-tube some milk over night at ordinary room temperature, The next day heat the contents to boiling. Explain the result.

8). Place 10cc of milk in each of 5 test-tubes.

To No. 1 add 1/2 cc of very dilute HCl (10 drops of HCl to 50cc of water).

To No. 2 add 1/2 cc of 2% Na_2CO_3 solution.

To No. 3 add 1/4 cc of saturated $(NH_4)_2C_2O_4$ solution (1-20)

Then add to each of these three tubes and also to Nos. 4 and 5 2 drops of rennet solution and mix. Heat the contents of tube No. 5 to boiling. Then place all the tubes in a water bath at 40° and examine every 3-5 minutes.

The contents of tube 1 will coagulate in a few minutes; No. 4 next, Nos. 2, 3, and 5 will not coagulate. The latter does not because the heat has destroyed the ferment. The action of rennet is retarded or prevented by the alkalis, and is favored by the acids, such as is present in the gastric juice.

The coagulum which forms contains paracasein and the fat. The clear liquid that separates from the coagulum on standing is the whey or milk serum. Paracasein is different chemically from the casein obtained by the addition of an acid to milk. Calcium salts must be present in order that paracasein may form Tube No. 3 does not coagulate because the calcium is thrown out of solution as the oxlate. Compare the change that takes place with that in the clotting of blood. If oxlate sodium is added to freshly drawn blood what is the result?

Continue heating tube 3 at 40° for about 1/2 hour. Then add 2-3 drops of $CaCl_2$ solution. The liquid instantly solidifies. This shows that the rennet has acted on the casein and changed into the modification which, with calcium, yields paraca

Calcium is likewise necessary to the coagulation of blood, not however, for the formation of clot directly as in the case of the milk curd. Calcium-free blood plasma (oxlate plasma) and calcium-free fibrin ferment when mixed, promptly yield a clot of fibrin. The calcium is necessary to the formation of the fibrin ferment from a parent substance, prothrombin (Hammersten).

9). To some milk in a test-tube add 1-2 volumes of ether, close and shake thoroughly. The fat globules do not dissolve; the milk remains opaque. Now add a few drops of NaOH and shake again. The ether now dissolves the fat and the liquid clears up. This reaction was taken at one time to indicate that the globules were surrounded by a albuminous envelop. Compare this test with the action of ether on blood.

10). To some milk in a test-tube add a few drops of NaOH and heat. The liquid becomes yellow, then orange and finally brown.

11). To a 4% solution of lactose add a little NaOH and test. The same color reaction is develpped aas in Exp. 10 which is due to the sugar present in the milk.

12). To some milk add a tincture of guajac and mix; then pour on a layer of old terpentine. A deep blue color develops. This test is also given by blood.

13). Repeat the prededing Exp. using, however, the boiled milk from Exp. 5. The color does not develop. Heat has changed the proteids so that they can no longer assist in the oxidation of the guajac resan.

14). To about 10cc of milk in a test0tube add 5g of powdered $MgSO_4$ and shake thoroughly. Then pour into a filter resting in a test-tube and filter over night. Boil the clear filtrate--albumin coagulates. The casein is precipitated almost completely my $MgSO_4$.

15). To 5cc of milk add 4 volumes (20cc) of strong alcohol shake thoroughly and set aside. All the proteids present are precipitated.

16). Dilute 10cc of milk with about 30cc of water and divide into three portions.

To 1 add 1-2cc of potassium alum solution (1-10) and shake. The casein is precipitated and carries down with it the fat.

To 2 add 1-2 cc of copper sulphate solution (1-10) and shake. A voluminous greenisn blue precipitate of the proteids present results.

To 3 add about 2 cc. of Almen's tannic acid solution and shake. The proteids are precipitated.

17). Moisten a few granules of pepsin with a drop of water, or better with a drop of a 0.7% NaCl solution. Then add 5 cc. of milk, mix and set aside in a water-bath at 40° . Coagulation results in a few minutes. It will fail if more water or salt solution is added to the pepsin (Pepelharing). Chymosin on digestion with pepsin and 0.3% HCl is destroyed (Hammersten).

18). Add 50 cc. of milk to about 400 cc. of water, mix well and while stirring add dilute acetic acid (1-10), drop by drop, till the precipitate becomes coarsely flocculent and ceases to increase . Stir thoroughly and set aside over night. The reaction should be distinctly acid.

The precipitate consists of casein and fat. Filter off the precipitate and allow to drain well, then fold over half the filter in the funnel and apply gentle pressure with the fingers until no more water can be squeezed out.

Transfer the precipitate to a small dry beaker add about 30 cc. of strong alcohol and stir thoroughly so as to dehydrate the casein. Then filter and again squeeze the contents of the filter as dry as possible. Transfer the precipitate to a small dry beaker, add about 50 cc. of ether and heat in a warm water-bath with constant stirring for about ten minutes. Owing to danger the light should be very low or better turned out. Finally transfer the contents to a filter and squeeze as dry as possible.

Spread open the filter on the table, allow the remaining ether to evaporate, then powder. The white chalky powder is casein.

The ether filtrate received in a small beaker on evaporating dish and evaporated cautiously on the water-bath gives the milk-fat.

The aqueous filtrate from the casein and fat precipitate contains albumin and milk-sugar. Place it in a beaker and boil for 15 minutes. Filter off the precipitate of albumin and reserve for subsequent tests.

Concentrate the filtrate from the albumin in a beaker on a wire gauze till it becomes cloudy and bumps. Cool the liquid, the cloudiness disappears and is therefore due to phosphates. Heat again to boiling and filter hot. Concentrate the filtrate now on the water-bath to a syrupy consistency and set aside over night. Crystals of milk-sugar separate on standing.

To the casein obtained in the above, with the biuret apply the millon and xanthoproteic reattions. Also dissolve a portion in water to which some Na_2CO_3 solution has been added. Observe the cloudiness of the solution. Heat a portion of the casein with alkaline lead acetate. What is the result? Apply the same tests to specimen of albumin. Review carefully the reactions for lactose and fats.

CHAPTER X.

MILK ANALYSIS.

The milk to be analyzed should be thoroughly shaken just before each portion is taken for anaalysis in order to insure a true sample. The quantity to be taken is measured out by means od a clean and dry 10cc pipette graduated in 1/10cc. The quantity taken multiplied by the specific gravity of the milk gives the weight of the milk employed for the determination.

1). SPECIFIC GRAVITY.

Determine the specific gravity of the specimen by means of--

a). The picnometer or specific gravity bottle. This is done according to directions given.

b). The lactometer.--There are two forms of this instrument commonly in use. The Suevenne-Müller lactometer, employed largely in Europe, gives the specific gravity direct. The lactometer of the New York Board of Health reads from 0°, the density of water at 15° which corresponds to 1.0, to 120° which represents a specific gravity of 1.0348. 100° on this scale represents the specific gravity of 1.029 which is taken as the minimum density of genuine milk. In the absehce of a lactometer the ordinary urinometer may be used although the divisions are very small and the reading consequantly is not accurate.

Place a sample of the milk in a suitable cylinder or 50cc graduate and determine the density.

Determine the specific gravity of the skimmed milk obtained from the following experiment. What is the result?

What is the affect of the addition of water to milk? To skimmed milk?

2). Creamometer.--Fill a 50cc graduate to the mark with milk. Set aside for 24 hours at the ordinary room temperature. Note the volume of the cream and calculate the volume per cent. A good milk should give 10-12 per cent of cream.

Remove the skimmed milk from below the layer of cream by means of a pipette and determine the density according to 1b.

3). Total solids.--Place 2cc of milk in a previously weighed watch-glass and evaporate on the water-bath to dryness. Then wipe the bottom of the watch-glass and place in an air-bath at 100-105° for 3 hours. Cool in dessicator and weigh rapidly. Calculate the per cent of total solids. This result subtracted from 100 gives per cent of water.

4). Ash.--Place 5cc of milk in a previously weighed porcelain crucible, evaporate to dryness on the water-bath. Then carefully ignite so as the char the mass slowly and thus avoid spurting. The ignition must be continued till the ash is grayish-white, free from carbon. Cool in desiccator and weigh. Calculate the per cent of ash.

5). Fat.--Roll up in a coil a strio of thick filter paper about 2 inches wide and 24 inches long, and tie it with a thresd or wire. 5cc of milk is allowed to run slowly on to one end of the coil. The coil, dry end down, is placed on a watch-glass and dried in an air-bath at 100-105° for one hour. It is then placed in a Loxhlet extraction apparatus which is connected by means of sound well fitting corks to an inverted condenser above and to a 150cc wide neck, round flask, or Erlenmeyer flask below. The weight of the clean dry flask is first ascertained. By means of a small funnel pour ether into the apparatus from above, until it siphons; then add about half as much ether. The flask is now heated, cautiously, on a water-bath so that the siphon will act about every five minutes. The extraction will be complete in from 1-1 1/2 hours. Then remove the paper coil and continue heating till the ether fills the extraction apparatus.and is almost ready to siphon. Disconnect the flask and transfer the ether from the apparatus to a bottle. The flask still contaens some ether, also water and fat. Heat on the water-bath to dryhess, wipe the flash carefully, and finally dry, in an air-bath at 100-105° for one hour. Cool in desicator and weigh. Calculate per cent. of fat. Subtract this result from that of total solids--difference is Solids not fat.

In manipulating with ether great care must be taken to prevent accidents. The corks must be large enough to fit snugly and the gas must be turned off until everything is in readiness to begin the extraction. To obtain very accurate results the coil of paper should be first extracted with ether to remove what fat may be present.

6). Lactose.--Place about 380cc of water in a beaker, add 20cc of milk qnd mix thoroughly. Then aid gradually about 2cc of dilute acetic acid,(1-10), with constant stirring, till a flocculent precipitate forms. The reaction should be destinctly acid. Place beaker on a wire gause and heat to boiling for 1/2 hour. Then filter thorugh a wet filter. Rince the beaker several times with hot water, and finally wash the residue on the filter, proteids and fats, with hot water. Concentrate the combined filtrate and wash water to about 150cc. Cool and measure the volume of liquid. Determine the lactose in this solution with Fehling's

solution according to the method described.

10cc of Fehling's solution corresponds to 0.067g of milk sugar.

Calculate the per cent. of lactose.

7). Casein.--To 50cc of water in a small beaker add 10cc of milk and mix. Warm on the water-bath to 40°. Then add 2 1/2 cc of potassium alum solution (1-10) and stir thoroughly. A finely flocculent precipitate should settle rapidly and the liquid should be clear. Let stand for about 15 minutes at 40°, then filter. If the filtrate is cloudy pass it again through the filter. Wash several times with water. Reserve the combined filtrate and wash-water for the next determination.

Place the filter with its contents in a Kjeldahl flask of about 250cc capacity, add 15cc H_2SO_4, 1/4 g powdered $CuSO_4$ and heat on wire gauze under the hood, till foaming ceases, then add 10g of powdered K_2SO_4 and continue gentle boiling till the liquid is light green. Finally add a little powdered $KMnO_4$, on the point of a knife, in order to complete the oxidation and heat till the liquid is light green in color. Allow to cool, then transfer the contents to a 1 , liter Erlenmeyer flask. Rince out the digesting flask several times with water and add this to the acid solution Dilute the contents of the flask to about 500cc and cool. Add a little powdered talc on the end of a knife. Insert in the neck of the flask a double perforated stopper rubber, provided with a Reitmaier bulb and a thistle tube. The end of the laster should reach nearly to the bottom of the flask. A long strip of red litmus paper should be suspended from the neck of the flask and should extend down into the liquid. Connect the free end of the Reitmaier bulb with a condenser. The lower end of the condenser is connected with a bent tube which extends down into the liquid of the receiving flask. This flask should be about 250cc capacity and contains 50cc of n/10 oxalic acid, which will unite with the NH_3 that will be distilled off. When all is in readiness pour into the flask, though the tube, strong NaOH solution (1-2) until the liquid is decidedly alkaline. About 50-60 cc. will be required Heat the large flask and distil over about 300 cc. Then replace the receiver by a flask containing 10 cc. of n/10 oxalic acid and some water and continue the distillation till about 100 cc. of distillate passesover.

To each of the receivers now add a few drops of alcoholic rosolic acid and titrate with n/10 NaOH to a strong pink reaction. The second flask serves as a check and should be free, or nearly free from ammonia. The difference between the number of cc. of oxalic acid employed and the number of cc. of n/10 NaOH necessary to neutralize the distillate gives the number of cc. of n/10 NH_3 given off in the distillation.

A blank experiment with filter paper, 15 cc. of H_2SO_4 and salts as above should be carried through in exactly the same manner to ascertain how much NH_3 may be given off by the reagents themselves. The number of cc. of n/10 NH_3 thus found should be subtracted as a

correction from the total number of cc. of n/10 NH_3 given off in the above. The difference multiplied by the n/10 factor of nitrogen 0.0014, gives the amount of nitrogen contained in the casein precipitate from 10 cc. of milk. This amount of nitrogen multiplied by 6.37 gives the amount of casein in 10 cc. of milk. Calculate the per cent. of casein.

8). GLOBULIN AND ALBUMIN.--

The filtrate from the alum precipitate of casein in the preceeding experiment contains globulin and albumin. To this filtrate add 10 cc. of Almen's tannic acid solution. Filter off the voluminous precipitate of proteids, wash several timeswith water, and allow to drain. Place the filter and convents in a Kjeldahl flask and determine the ni trogen as above, making the proper correction for blank. The amount of nitrogen found multiplied by the factor 6.37 gives the amount of albumin and globulin in 10 cc. of milk, Calculate the per cent. of albumin and globulin.

Amen's tannic acid solution is prepared according to the following formula: Tannic acid 4 g.; 8 cc of 25% acetic acid; 90 cc. of 90% alcohol; 100 cc. of water.

9). TOTAL NITROGEN IN MILK.--

Place 10 cc. of milk in a Kjeldahl flask add 15 cc. of H_2SO_4 and treat as above under exp. 7. This gives the total nitrogen present in the milk and serves as a control on the two preceeding determinations.

A report of the results obtained is to be made out, as follows; together with a statement as to whether the milk is adulterated or not:

1. Specific gravity, whole milk . . . . .
1. Specific gravity, whole milk, ...
2. Specific gravity, skimmed milk, ...
3. Cream, per cent. of, ...
4. Water, " " " . . . . . .
5. Total solids " " . . . . . .
6. Total solids not fat, per cent of, . . .
7. Fat, per cent of, ...
8. Ash, " " " . . . . . .
9. Lactose, " " . . . . . .
10. Casein " " . . . . . .
11. Globulin and Albumin, per cent of, . . .
12. Total nitrogen as proteid, per cent of. .

To decide upon the purity of a milk the determination given under (4), 1, (4), 5, (6), 7, are as a rule sufficient. In case of doubt the ash may be determined. The legal standard of milk varies in different states. In New York the minimum of total solids allowed is 12%; of fat 3 %. In Massachusetts the total solids must not fall below 13%. New Jersey allows a minimum of 12% for total solids.

The following table, compiled from König, shows the average percentage composition of various milk:

Milk of:	No. of analyses averaged:	Specific Gravity:	Water:	Casein:	Albumin:	Fat:	Lactose:	Ash.:
Woman	107	1.027	87.41	1.03	1.26	3.78	6.21	0.31
Cow	793	1.0315	87.17	3.02	0.53	3.69	4.88	0.71
Cow, (Colostrum)	42	----	74.67	4.04	13.60	3.59	2.67	1.56
Goat	38	----	85.71	3.20	1.09	4.78	4.46	0.76
Sheep	32	1.034	80.82	4.79	1.55	6.86	4.91	0.89
Mare	47	1.0347	90.78	1.24	0.75	1.21	5.67	0.35
Ass	4	-----	89.64	0.67	1.55	1.64	5.99	0.51
Hog	7	-----	84.04	7.23		4.55	3.13	1.05
Dog	28	-----	75.44	6.10	5.05	9.57	3.09	0.73

--==:: THE END ::==--

APPENDIX.

Ehrlich's Diazo-reaction.

The reagent employed for this reaction should be freshly prepared. Two solutions are first prepared.

(1)—To 1000 C.C. of water add 50 C.C. of H Cl and 5 g. of Sulphanilic Acid.

(2)—A 0.5% solution of Sodium Nitrite.

Just before use these two solutions can be mixed to form the reagent proper as follows: To 250 C.C. of solution No.1 add 5 C.C. of solution No. 2. Or, on a smaller scale, to 5 C.C. of No. 1 add 3/4 drops of solution No. 2. The Nitrite solution is subject to oxidation on standing, and should not therefore be prepared in large quantity.

Mix the urine with an equal volume of the reagent, and add at once an excess of N H_4O H . A pink to a deep red color, and especially a pink colored foam, constitutes the diazo-reaction.

Normal urine as a rule gives a brownish yellow, very rarely a pinkish color. The reaction is very rare in Chronic non-febrile diseases. It is met with as a rule, excepting in very light cases, in typhoid fever, and a certain diagnostic value is therefore ascribed to this reaction. It has been found however, in exanthemic typhus, in smallpox, in acute initiary tuberculosis, in severe tuberculosis, and in pneumonia. The dissapearance of the reaction in typhoid urine may be taken as a favorable sign, while the appearance of the reaction in tuberculosis is an unfavorable sign.

The substance which gives this reaction is unknown. It is an aromatic compound, probably a metabolic product which appears in the urine only under certain special conditions.

The reaction resembles somewhatthe test for nitrites as given in experiment 11 under Saliva. If nephthylamine is replaced by A-Naphthol the reaction is even more similar.

APPENDIX II.

Estimation of Alloxuric Bodies and Bases.

Uric acid and the nuclein bases are known to possess an alloxan group and an urea group in their molecule. They are moreover all precipitated on boiling with a solution of Copper Sulphate and a reducing agent. The term Alloxuric bodies has been given to all those constituents of urine which contain the two groups mentioned. Deducting from the Alloxuric bodies the uric acid present in the urine, leaves the alloxuric bases. The latter therefore includes the nuclein bases, as well as other related compounds which have not as yet been isolated from the urine.

The reagents employed are a 13% solution of Copper Sulphate; a solution of Sodium acid Sulphite (1—2); and a 10% solution of Barium Chloride. The method is as follows:

Place 100 C.C. of the Albumin-free urine in a beaker and boil, then add 10 C.C. of the Copper Sulphate solution and 10 C.C. of the Sodium acid Sulphite solution and boil. Later add 5 C.C. of the Barium Chloride solution in order to cause the precipitate to settle more readily. Let stand two hours. Then transfer to a small pleited filter and wash five times with water heated to 60°. Then place the filter and contents in a Kjeldahl flask, and determine the Nitrogen present according to Kjeldahl's method as given under Milk.

A blank experiment must be made, using a clean filter paper, instead of the one with the precipitate. The number of C.C. of decinormal Ammonia which is found in this blank experiment must be deducted from the number of C.C. found in the above experiment. The difference represents the number of C.C. of decinormal Ammonia formed from the Alloxuric bodies in 100 C.C. of the urine. Therefore, this number of C.C. multiplied by the decinormal factor of Nitrogen gives the Nitrogen in the Alloxuric Bodies.

In another sample of the urine determine the amountof the uric acid according to the Salkowski-Ludwig method as given in the book. Calculate the amount of the uric acid as contained in 100 C.C. of the urine, and then the amount of Nitrogencontained in the uric acid present in 100 C.C. of urine.

Subtracting the found Nitrogen of uric acid from the found Nitrogen of Alloxuric bodies gives the Nitrogen of Alloxuric Bases.

The ratio of the Nitrogen of alloxuric bases to the Nitrogen of uric acid is about 1--4 in normal urine. In laukamia it may rise to 1--1.

Determination Of Total Nitrogen To Urine.---

Place 5 C.C. of urine in a Kjeldahl flask and determine Nitrogen present according to Kjeldahl's method. Receive the distillate in 50 C.C. of decinormal oxalic acid.

APPENDIX III.

Quantitative Analysis of Gastric Juice.

TOEPFER'S METHOD.----The following reagents are necessary:

(1)--Decinormal Sodium Hydrate solution.

(2)--A 1% Alcoholic solution of Phenol-phthalein. This indicates total acids.

(3)--A 1% aqueous solution of Sodium olizainsulphonic acid. This indicates all acids except the loosely combined.

(4)--A 0.5% Alcoholic di-methyl amidoazobenzol solution. This indicates only free HCl.

The method is as follows: Measure out into 3 beakers 10 C.C. of the filtered Gastric Juice. If necessary a smaller amount may be taken, or the Gastric Juice may be diluted.

To beaker No. 1 add 1—2 drops phenol phthalein, then run in decinormal Sodium Hydrate, not to the first pink color, but to a dark red c. which no longer increases in depth. Note the number of C.C. of reagent employed.

To beaker No. 2 add 3—4 drops of the Alizarin solution and then titrate with decinormal Sodium Hydrate until the first pure violet color is reached. Note the number of C.C. employed.

To beaker No. 3 add 3—4 drops of di-methyl amidoazobenzol solution. A yellow color indicates the absence of free H Cl. If a red color is present run in decinormal Sodium Hydrate till it just disappears. Note the number of C.C. employed.

The difference between the number of C.C. of decinormal Sodium Hydrate required for beaker No. 1 (total acids) and the number of C.C. required for beaker No. 2 (all acids except loosely combined), gives the loosely combined H Cl.

The number of C.C. required for beaker No. 2 gives the free H Cl.

The number of C.C. required for beaker No. 1 gives the total Acidity.

The number of C.C. of reagent required for beaker No. 1 minus the number of C.C. required for beaker No. 2, minus the number of C.C. found to correspond to the loosely combined H Cl. gives the Organic Acids and Acid Salts.

APPENDIX IV.

The Mitscherlich Polarimeter.

The instrument consists of two Nicol prisms, the polarizer and analyzer, enclosed in brass tubes and supported in such a way that they can be rotated; the tube containing the analyzer has a pointer attached which measures the amount of its rotation upon a circle graduated in degrees. Between the two Nicols is placed the observation tube, a brass tube exactly 200 M.M. long, the ends of which are closed with glass plates; this holds the solution to be tested.

Adjust the instrument as follows:

Place a lamp behind the polarizer and fill the observation tube with distilled water; set the pointer at 0°, and then rotate the polarizer until the field becomes darkest. The polarizer must not be moved again.

As the instrument now stands, the two Nicols have their section at right angles. If the analyzer is rotated, one way or the other, the field gradually becomes brighter and is brightest when the pointer is at 90°, the sections of the prisms now being parallel. The field will be dark again at 180°.

Starting with the instrument adjusted as above, fill the observation tube with the solution to be tested. If this has the power of

rotating the plane of polarization, the field appears bright; The analyzer must now be turned to the right or left till the field again becomes darkest, thus compensating for the rotatory power of the solution. This shows whether the substance is dextro or laevo-rotatory. By knowing the length of the tube, the concentration of the solution, and the number of degrees through which the analyzer was turned, the Specific Rotatory Power can be calculated.

APPENDIX V.

The Soleil-Ventzke Saccharimeter.

This instrument is used only for the purpose of determining the percentage of cane sugar in a given sample.

It consists of two Nicol prisms, the analyzer and polarizer, and the observation tube placed between them. Between the polarizer and source of light is the regulator, a Nicol prism and a quartz plate, for the purpose of changing the colors. Between the analyzer and tube is the compensator: this consists of two wedge-shaped plates, one fixed, and the other capable of being slid over it, thus increasing or diminishing the thickness of the crystal through which the polarized ray passes. Fastened to the movable plate is a scale graduated so that it can be read to tenths of one percent; the reading is done by means of a vernier and telescope. The source of light is a lamp placed back of the polarizer.

With the scale reading at 0°, and the tube filled with distilled water, the field appears as a colored circle divided vertically, and both halves of exactly the same shade of color. This color may be changed by simply rotating the regulator. For most persons the "sensitive tint" is a rose violet.

Now fill the tube with a solution of cane sugar, prepared as given below. The plane of polarization is deviated, and the two disks are of different colors. Then turn the screw of the compensator till the disks are again of the same shade, thus compensating for the deviating effect of the sugar. The percentage of cane sugar can now be read distinctly from the scale.

The instrument is so made that with a solution of pure cane sugar containing 26.048 grms. in 100 C.C. at 17.5° C. the reading will be 100%. Consequently, in making a determination, dissolve 26.48 grms. of the substance in distilled water at 17.5°, and dilute to 100 C.C. The reading obtained will be the percentage of cane sugar in the substance.

APPENDIX VI.

PANCREAS.

The pancreatic secretion is a clear thick alkaline fluid, rich in solids and possess very active ferment properties. It contains at least three distinct frments besides albumen, leucin, fats, soap, and salts. These solid constitients make up about 10% of the secretion. After a pancreatic fistula has been in place for sometime the secretion is altered. It becomes thiner, strongly alkaline and shows little or no proteclytic action. The amount of solids in this altered secretion scarcely exceeds 2 per cent. The quantity of the secretion given off in a period of 24 hours is not definitely known.

The ingestion of food stimulates the flow of the gastric juice.. There is, therefore, no secretion during starvation and in carniverous animals where some time elapses between meals it is intermittent. On the other hand the secretion is going on almost continually in herbivorous animals because digestion is uninterruptedly taking place.

As stated above the pancreatic secretion contains at least three distinct ferments or enzymes splitting up respectively fats, carbohydrates and proteids.

The neutral fat which is taken into the body with the food is acted upon by one of the ferments steapsin or pialyn and is split up by hydration saponification into free fatty acids and glycerin. This ferment is very readily decomposed by acids and may be absent therefore from old pancreas. Only a small portion of the fat, however, undergoes this change. The free acids now combine with sodium carbonate to form soaps and the resulting soap solution readily emusifies the remaining neutral fat and thus brings it into a finely divided condition suitable for absorption. A considerable portion of the fat , at times, be decomposed into free fatty acids through the activity of bacteria. The free fatty acids are not absorbed as such, but appear to be regenerated in the intestinal walls, by synthein, into neutral fat. Only a very small amount of fat seems to be absorbed as soap:

The cleavage of fats by the pancreatic ferment and the subsequent emulsification is necessary to the proper absorption of fats. In addition to the pancreatic secretion, the bile plays and important part in the absorption of fat. It is well known that closure of the bile-duct, whether experimentally, or in disease as in icturus, is followed by diminished absorption of fat and increased excretion of fat, more especially fatty acids, in the feces. Some part, however, continues to be absorbed even in the absence of the bile secretion. The pancreatic secretion, however, is necessary since no absorption of fat takes place when the pancreas is extirpated. In the latter case, however, milk continues to be absorbed owing to the already emulsified condition of the fat. Some fat may,at times , be absorbed even after total extirpation of the pancreas since bacterial ferments may split up the fat and thus emulsification and hence absorption may result.

The second ferment of the pancreas acts on starches splitting up the bodies into dextrin and iso-maltose. This ferment is spoken of as amylolytic or diastatic and resembles in its action the ptyalin of the saliva. It is probably not identical with the saliva ferment. It is

soluable in water, and in glycerin; insoluable in alcohol. This diastatic ferment appears to be absent during the first few weeks of infant life. At the temperature of the body it acts rapidly on boiled starch, converting this into amylodextrin, erythrodextrin, achroodextrin, iso-maltose and maltose. By the action of a special inverting ferment the maltose then is converted into glucose in which form the carbohydrates are chiefly absorbed. Other mono-saccharides as laevulose and galactose may also be absorbed direct. It is possible for small amounts of dextrin and for milk sugar to reach absorption.

Sugar is absorbed very rapidly so much so, indeed, that if a very large amount be ingested at one time it appears in the urine. This condition known as alimentary glycosuria, does not accur when large quantities of starch are ingested. Although the pancreatic gland is necessary to the complete absorption of all the starch ingested, it is a note-worthy fact that about one-half of the starch ingested will still be absorbed after total extirpation of the pancreatic gland. This may be explained by the diastatic action possessed by many bacteria.

The third ferment of the pancreatic secretion is proteolytic in its action and is known as trypsin. This ferment does not exist as such in the substance of the gland but is represented by a parent-substance trypsinogen which is most abundant in the gland in from 14-18 hours after a meal. This zymogin during the process of secretion is converted into enzyme trypsin. Just how this takes place is not definitely known. This conversion con be accomplished artificially by the action of air, water, acids, very weak alkalis and various other substances. It is probable that, as in the case of pepsin, the pancreatic secretion of different animals contains slightly different trypsins. Stronger alkalis present the cleavage of the zymogen.

Trypsin, like other ferments, in its purest condition proteid reactions. It is soluable in wat er, insoluable in alcohol and glycerin. When in an impure state, however, it may be dissolved by glycerin. This is true of the other enzymes. In neutral or slightly alkaline solution it is readily destroyed at 50 degrees. It is also destroyed by gastric juice and unlike pepsin it digests fibrin in alkaline, neutral or even very fainly acid solutions. It is destroyed by mineral acids but not as a rule by organic acids. The fibrin in tryptic digestion does not swell and is not irregularly eaten away as is the case in peptic digestion. The fibrin digestion with trypsin takes place most rapidly at about 40 degrees and in slightly alkaline solution (0.3% Na_2Co_3).

In view of the fact that trypsin acts best under the conditions mentioned, it is evident that the products of the tryptic digestion will be mixed with various bacterial products unless special attention is given toward inhibiting the growth of these micro-organisms.

In actual experiments therefore thymol or chloroform is added to suppress the bacteria. In the intestines, of course, during pancreatic digestion the bacteria are unhindered in teir action. Among the products resulting from the action of trypsin proper, on fibrin may be mentioned albumoses, pepton, leucin, tyrosin, asparaginic acid, lysin, ammonia and proteinochromogen. True pepton is formed much more readily in tryptic than in peptic digestion. This pepton is eventually of the kind known as antipepton, whereas the hemipepton has been decomposed yielding products such as leucin, tryosin etc. Trypsin dissolves gelatin yielding a gelatin-pepton. The collagens or gelatin-yielding connective tissues are not acted upon until they have been altered by heat or acids. Trypsin has no action on fats or carbohydrates.